

10-16-00

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Customer No. 000959

Case Docket No. AVZ-007CP3

10/13/00

jc956 U.S. PTO

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jc920 U.S. PTO
09/687575
10/13/00

Sir:

Transmitted herewith for filing is the continuation-in-patent application of

Inventor(s): Rima Kaddurah-Daouk and M. Flint Beal

For: COMPOSITIONS CONTAINING A COMBINATION OF A CREATINE COMPOUND AND A SECOND AGENT

Enclosed are:

- ☒ This is a request for filing a continuation-in-part application of pending prior application serial no. 09/285,395 filed on April 2, 1999 entitled Compositions Containing a Combination of a Creatine Compound and a Second Agent, which in turn is a continuation-in-part patent application of serial no. 09/283,267, filed on April 1, 1999.
- ☒ 60 pages of specification, 12 pages of claims, 1 pages of abstract.
- ☒ 7 sheets of informal drawings (Figures 1-7).
- ☒ An unexecuted Declaration, Petition and Power of Attorney (6 pages).
- ☐ An assignment of the invention to _____ A recordation form cover sheet (Form PTO 1595) is also enclosed.
- ☐ A verified statement to establish small entity status under 37 C.F.R. 1.9 and 37 C.F.R. 1.27.
- ☐ Other _____

The filing fee has been calculated as shown below:

	(Col. 1)	(Col. 2)
FOR:	NO. FILED	NO. EXTRA
BASIC FEE	////////////////////	
TOTAL CLAIMS	63 - 20	= 43
INDEP. CLAIMS	6 - 3	= 3
<input type="checkbox"/> MULTIPLE DEPENDENT CLAIMS PRESENTED		

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SMALL ENTITY	
RATE	FEE
////////	\$ 355.00
x 9=	\$ 387.00
x 40	\$ 120.00
+135	\$
TOTAL	\$862.00

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RATE	FEE
////////	\$
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TOTAL	0

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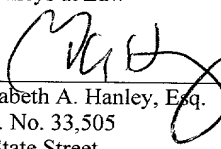
09/687575 10/13/00

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- ☐ A check in the amount of \$ _____ to cover the recording of assignment documents is also enclosed.
- ☒ Address all future communications (May only be completed by applicant, or attorney or agent of record) to Elizabeth A. Hanley, Esq. at **Customer Number: 000959** whose address is:

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Date: October 13, 2000

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COMPOSITIONS CONTAINING A COMBINATION OF A CREATINE COMPOUND AND A SECOND AGENT

Related Applications

This application is a continuation-in-part of U.S. Patent Application Serial
10 No. 09/285,395, entitled "Compositions Containing a Combination of a Creatine
Compound and a Second Agent," filed on April 2, 1999; which is a continuation-in-
part of U.S. Patent Application Serial No. 09/283,267, entitled "Compositions
Containing a Combination of a Creatine Compound and a Second Agent," filed on
April 1, 1999; and claims priority to U.S. Provisional Application Serial No.
15 60/080,459, entitled "Compositions Containing a Combination of a Creatine
Compound and a Second Agent," filed on April 2, 1998; the entire contents of each
of the aforementioned applications are hereby incorporated herein by reference. The
application is related to U.S. Provisional Application Serial No. 60/XXX,XXX,
entitled "Compositions Containing A Combination of a Creatine Compound and a
20 Second Agent," filed on October 13, 2000, the entire contents of which are hereby
incorporated herein by reference. The entire contents of each of PCT/US95/14567,
filed November 7, 1995, U.S. Serial No. 08/336,388, filed November 8, 1994 and
U.S. Serial No. 08/853,174, filed May 7, 1997 are also hereby incorporated herein by
reference.

25

Background of the Invention

Creatine is a compound which is naturally occurring and is found in
mammalian brain and other excitable tissues, such as skeletal muscle, retina and
heart. Its phosphorylated form, creatine phosphate, also is found in the same organs
30 and is the product of the creatine kinase reaction utilizing creatine as a substrate.
Creatine and creatine phosphate can be synthesized relatively easily and are believed
to be non-toxic to mammals. Kaddurah-Daouk et al. (WO 92/08456 published May
29, 1992 and WO 90/09192, published August 23, 1990; U.S. 5,321,030; and U.S.
5,324,731) describe methods of inhibiting the growth, transformation and/or
35 metastasis of mammalian cells using related compounds. Examples of compounds
described by Kaddurah-Daouk et al. include cyclocreatine, b-guandidino propionic
acid, homocyclocreatine, 1-carboxymethyl-2-imino-hexahydropyrimidine, guanidino
acetate and carbocreatine. These same inventors have also demonstrated the efficacy
of such compounds for combating viral infections (U.S. 5,321,030). Elgebaly in U.S.
40 Patent 5,091,404 discloses the use of cyclocreatine for restoring functionality in
muscle tissue. Cohn in PCT publication No. WO94/16687 described a method for
inhibiting the growth of several tumors using creatine and related compounds.

Neuroprotective agents can be found in nature and help to maintain an organisms ability to function without general distress to the nervous system. Often times, reduced levels below what is considered "normal" for these agents, can lead to diminished function of the nervous system.

The nervous system is an unrelenting assembly of cells that continually receives information, analyzes and perceives it and makes decisions. The principle cells of the nervous system are neurons and neuroglial cells. Neurons are the basic communicating units of the nervous system and possess dendrites, axons and synapses required for this role. Neuroglial cells consist of astrocytes, oligodendrocytes, ependymal cells, and microglial cells. Collectively, they are involved in the shelter and maintenance of neurons. The functions of astrocytes are incompletely understood but probably include the provision of biochemical and physical support and aid in insulation of the receptive surfaces of neurons. In addition to their activities in normal brain, they also react to CNS injury by glial scar formation. The principle function of the oligodendrocytes is the production and maintenance of CNS myelin. They contribute segments of myelin sheath to multiple axons.

The ependyma cells react to injury mainly by cell loss. Microglial cells become activated and assume the shape of a macrophage in response to injury or destruction of the brain. These cells can also proliferate and adopt a rod-like form which could surround a tiny focus of necrosis or a dead neuron forming a glial nodule. Microglial degradation of dead neurons is called neuronophagia.

The creatine kinase/creatine phosphate energy system is only one component of an elaborate energy-generating system found in nervous system cells such as, for example, neurons, oligodendrocytes and astrocytes. The components of the creatine energy system include the enzyme creatine kinase, the substrates creatine and creatine phosphate, and the transporter of creatine. The reaction catalyzed by creatine kinase is: $\text{MgADP} + \text{PCr}^- + \text{H}^+ \rightarrow \text{MgATP}^- + \text{Cr}$. Some of the functions associated with this system include efficient regeneration of energy in cells with fluctuating and high energy demands, energy transport to different parts of the cell, phosphoryl transfer activity, ion transport regulation, and involvement in signal transduction pathways.

5 The creatine kinase/phosphocreatine system has been shown to be active in
neurons, astrocytes, oligodendrocytes and Schwann cells. Manos et al., *J.*
Neurochem. 56:2101-2107 (1991); Molloy et al., *J. Neurochem.* 59:1925-1932. The
activity of the enzyme has been shown to be up-regulated during regeneration and
down-regulated in degenerative states (see, e.g., *Annals Neurology* 35(3):331-340
10 (1994); DeLeon et al., *J. Neurolsci. Res.* 29:437-448 (1991); Orlovskaya et al.
Vestnik Rossiiskoi Akademii Meditsinskikh Nauk. 8:34-39 (1992). Burbaeva et al.,
Shurnal Neuropathologii Psikiatrii Imeni S-S-Korsakova 90(7):85-87 (1990);
Mitochondrial creatine kinase was recently found to be the major constituent of
pathological inclusions seen in mitochondrial myopathies. Stadhouders et al., *PNAS*
15 91:5080-5093 (1994).

 It is an object of the present invention to provide methods for treatment of
diseases that affect cells of the nervous system that utilize the creatine
kinase/phosphocreatine system using compounds which modulate the system.

20

Summary of the Invention

 The present invention is based, at least in part, on the discovery that certain
combinations of creatine compounds and neuroprotective agents, described *infra*, can be
used to treat a nervous system disease. Examples of such disease include those which
25 there is undesired neuronal activity, characterized by undesirable demyelinating,
dysmyelinating or degenerative neuronal activity in a mammal. Compositions and
methods of the invention include combinations of creatine compounds and
neuroprotective agents. Preferred creatine compounds include creatine, creatine
phosphate, cyclocreatine, cyclocreatine phosphate, beta guanidino propionic acid, and
30 combinations thereof. Preferred neuroprotective agents include: approved drugs for the
treatment or prevention of neurodegenerative diseases such as Riluzole, Cognex,
Aricept, Sinmet, Sinmet CR, Permax, Parlodel, Elepryl, Symmetrel, Artane); glutamate
excitotoxicity inhibitors (such as glutamate uptake and biosynthesis modulation with
compounds like gabapentin and Riluzole); growth factors like CNTF, BDNF, IGF-1;
35 nitric oxide synthase inhibitors; cyclo-oxygenase inhibitors such as aspirin; ICE
inhibitors; Neuroimmunophilins; N-acetylcysteine and procysteine; antioxidants (such as
pyruvate and lutein), energy enhancers (such as ribose and vincopocetine), vitamins and
cofactors (such as spin traps, CoQ₁₀, carnitine, nicotinamide, Vitamin E or D) lipoic acid,
vinpocetine, other fatty acids (such as docosahexanoic acid (DHA), eicosapentenoic acid
40 (EPA), and gamma linolenic acid (GLA)), various herbal extracts (such as rosemary and

5 2) an aryl group selected from the group consisting of: a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents independently selected from the group consisting of: $-CH_2L$ and $-COCH_2L$ where L is independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy; and

10

3) -NH-M, wherein M is selected from the group consisting of: hydrogen, C₁-C₄ alkyl, C₂-C₄ alkenyl, C₁-C₄ alkoyl, C₃-C₄ branched alkyl, C₃-C₄ branched alkenyl, and C₄ branched alkoyl;

15 c) X is selected from the group consisting of NR₁, CHR₁, CR₁, O and S,
wherein R₁ is selected from the group consisting of:

1) hydrogen;

20 2) K where K is selected from the group consisting of: C₁-C₆
straight alkyl, C₂-C₆ straight alkenyl, C₁-C₆ straight alkoyl, C₃-C₆ branched alkyl,
C₃-C₆ branched alkenyl, and C₄-C₆ branched alkoyl, K having 0-2 substituents
independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

25 3) an aryl group selected from the group consisting of a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents independently selected from the group consisting of: $-CH_2L$ and $-COCH_2L$ where L is independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

30 4) a C₅-C₉ α-amino-ω-methyl-ω-adenosylcarboxylic acid attached
via the ω-methyl carbon;

5) a C₅-C₉ a-amino-w-aza-w-methyl-w-adenosylcarboxylic acid attached via the w-methyl carbon; and

35

6) a C₅-C₉ α-amino-ω-thia-ω-methyl-ω-adenosylcarboxylic acid attached via the ω-methyl carbon;

d) Z_1 and Z_2 are chosen independently from the group consisting of: =0, -NHR₂, -CH₂R₂, -NR₂OH; wherein Z_1 and Z_2 may not both be =0 and wherein R₂ is selected from the group consisting of:

5

1) hydrogen;

2) K, where K is selected from the group consisting of: C₁-C₆ straight alkyl; C₂-C₆ straight alkenyl, C₁-C₆ straight alkoyl, C₃-C₆ branched alkyl, C₃-C₆ branched alkenyl, and C₄-C₆ branched alkoyl, K having 0-2 substituents independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

3) an aryl group selected from the group consisting of a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents independently selected from the group consisting of: -CH₂L and -COCH₂L where L is independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

4) a C₄-C₈ α-amino-carboxylic acid attached via the α-carbon;

5) B, wherein B is selected from the group consisting of: -CO₂H, -NHOH, -SO₃H, -NO₂, OP(=O)(OH)(OJ) and -P(=O)(OH)(OJ), wherein J is selected from the group consisting of: hydrogen, C₁-C₆ straight alkyl, C₃-C₆ branched alkyl, C₂-C₆ alkenyl, C₃-C₆ branched alkenyl, and aryl, wherein B is optionally connected to the nitrogen via a linker selected from the group consisting of: C₁-C₂ alkyl, C₂ alkenyl, and C₁-C₂ alkoyl;

6) -D-E, wherein D is selected from the group consisting of: C₁-C₃ straight alkyl, C₃ branched alkyl, C₂-C₃ straight alkenyl, C₃ branched alkenyl, C₁-C₃ straight alkoyl, aryl and aroyl; and E is selected from the group consisting of: -(P(=O))_nNMP, where n is 0-2 and NMP is ribonucleotide monophosphate connected via the 5'-phosphate, 3'-phosphate or the aromatic ring of the base; -[P(=O)(OCH₃)(O)]_m-Q, where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base; -[P(=O)(OH)(CH₂)]_m-Q, where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base; and an aryl group containing 0-3 substituents chosen independently from the group consisting of: Cl, Br, epoxy, acetoxy, -OG, -C(=O)G, and -CO₂G, where G is independently selected from the group consisting of: C₁-C₆ straight alkyl, C₂-C₆ straight alkenyl, C₁-C₆ straight alkoyl, C₃-C₆ branched alkyl, C₃-C₆ branched alkenyl, C₄-C₆ branched alkoyl, wherein E may be attached to any point to D, and if D is alkyl or alkenyl, D may be connected at either or both ends by an amide linkage; and

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5 black caraway), and berry oils and meals (such as bilberry, elderberry, english hawthorn
berry, blackberry, blueberry, red and black raspberries).

The present invention further provides pharmaceutical compositions for modulating a nervous system disease in a subject. The pharmaceutical compositions include a synergistically effective amount of a combination of a creatine compound having the formula described above, a neuroprotective agent and a pharmaceutically acceptable carrier. In preferred embodiments, the creatine compound is creatine, creatine phosphate, cyclocreatine or cyclocreatine phosphate, beta guanidino propionic acid, and combinations thereof.

15 The present invention provides packaged nervous system disease modulators which include a creatine compound having the formula described above and at least one neuroprotective agent. Additionally, or in place of the neuroprotective agent, a creatine compound can be combined with existing therapeutic drugs for neurodegenerative

20 diseases.

Some of the diseases susceptible to treatment with creatine compounds according to the present invention include, but are not limited to Alzheimer disease, Parkinson's disease, Huntington's disease, motor neuron disease, diabetic and toxic neuropathies, traumatic nerve injury, multiple sclerosis, acute disseminated encephalomyelitis, acute necrotizing hemorrhagic leukoencephalitis, diseases of dysmyelination, mitochondrial diseases, fungal and bacterial infections, migrainous disorders, stroke, aging, dementia, and mental disorders such as depression and schizophrenia.

30 The present invention also provides compositions of creatine compounds, including the formula described above, and neuroprotective agents. Preferred creatine compounds include creatine, creatine phosphate, cyclocreatine or cyclocreatine phosphate, beta guanidino propionic acid, and combinations thereof. Preferred neuroprotective agents include : approved drugs for the treatment or prevention of
35 neurodegenerative diseases such as Riluzole, Cognex, Aricept, Sinmet, Sinmet CR, Permax, Parlodel, Elepryl, Symmetrel, Artane); glutamate excitotoxicity inhibitors (such as glutamate uptake and biosynthesis modulation with compounds like gabapentin and Riluzole); growth factors like CNTF, BDNF, IGF-1; nitric oxide synthase inhibitors; cyclo-oxygenase inhibitors such as aspirin; ICE inhibitors;
40 Neuroimmunophilins; N-acetylcysteine and procysteine; antioxidants (such as pyruvate and lutein), energy enhancers (such as ribose and vincopocetine), vitamins and cofactors

5 (such as spin traps, CoQ₁₀, carnitine, nicotinamide, Vitamin E or D) lipoic acid, vinpocetine, other fatty acids (such as docosahexanoic acid (DHA), eicosapentenoic acid (EPA), and gamma linolenic acid (GLA)), various herbal extracts (such as rosemary and black caraway), and berry oils and meals (such as bilberry, elderberry, english hawthorn berry, blackberry, blueberry, red and black raspberries).

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The present invention further provides compositions of creatine compounds, including the formula described above, and neuroprotective agents developed as a nutritional supplement, medical food or drug form. Preferred creatine compounds include creatine, creatine phosphate, cyclocreatine, cyclocreatine phosphate, beta guanidino propionic acid, and combinations thereof. Preferred neuroprotective agents include: approved drugs for the treatment or prevention of neurodegenerative diseases such as Riluzole, Cognex, Aricept, Sinmet, Sinmet CR, Permax, Parlodel, Elepryl, Symmetrel, Artane); glutamate excitotoxicity inhibitors (such as glutamate uptake and biosynthesis modulation with compounds like gabapentin and Riluzole); growth factors like CNTF, BDNF, IGF-1; nitric oxide synthase inhibitors; cyclo-oxygenase inhibitors such as aspirin; ICE inhibitors; Neuroimmunophilins; N-acetylcysteine and procysteine; antioxidants (such as pyruvate and lutein), energy enhancers (such as ribose and vincopocetine), vitamins and cofactors (such as spin traps, CoQ₁₀, carnitine, nicotinamide, Vitamin E or D) lipoic acid, vinpocetine, other fatty acids (such as docosahexanoic acid (DHA), eicosapentenoic acid (EPA), and gamma linolenic acid (GLA)), various herbal extracts (such as rosemary and black caraway), and berry oils and meals (such as bilberry, elderberry, english hawthorn berry, blackberry, blueberry, red and black raspberries).

30 Brief Description of the Figures

Figure 1 is a graph illustrating the effect of creatine and cyclocreatine on lesion volumes in mice using the malonate model.

Figure 2 is a graph illustrating the dose-response effects of creatine and
35 cyclocreatine on lesion volumes in mice using the malonate model.

Figure 3 is a graph illustrating the effect of creatine on lesion volumes in mice using the 3-NP model.

Figure 4 is a graph illustrating the effect of creatine and cyclocreatine on levels of dopamine, HVA, and DOPAC in mice using the MPTP model.

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Figure 5 is a graph illustrating the dose-response effects of creatine and cyclocreatine on levels of dopamine, HVA and DOPAC in mice using the MPTP model.

10

Figure 6 is a graph illustrating the effect of creatine in slowing the rate of motoneuronal degeneration of FALS mice.

Figure 7 is a graph illustrating the effect of creatine on improving the survival times of FALS mice.

15

Detailed Description

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The features and other details of the invention will now be more particularly described and pointed out in the claims. It will be understood that the particular embodiments of the invention are shown by way of illustration and not as limitations of the invention. The principle features of this invention can be employed in various embodiments without departing from the scope of the invention.

25

The methods of the present invention generally comprise administering to an individual afflicted with a disease of the nervous system a therapeutically effective amount of a creatine compound or compounds in combination with a neuroprotective agent or agents which modulate one or more of the structural or functional components of the creatine kinase/phosphocreatine system sufficient to prevent, reduce or ameliorate symptoms of the disease. Components of the system which can be modulated include the enzyme creatine kinase, the substrates creatine and creatine phosphate, and the transporter of creatine. As used herein, the term "modulate" means to change, affect or interfere with the functions of the creatine kinase system.

35

The present invention is based, at least in part, on the discovery that certain combinations of creatine compounds and neuroprotective agents, described *infra*, can be used to treat a nervous system disease. Examples of such diseases include those in which there is undesired neuronal activity, characterized by undesirable demyelinating, dysmyelinating or degenerative neuronal activity in a mammal. Compositions and methods of the invention include combinations of creatine compounds and neuronal modulatory agents. Preferred creatine compounds include creatine, creatine phosphate, cyclocreatine, cyclocreatine phosphate, beta guanidino propionic acid and combinations

5 thereof. Preferred neuroprotective agents include: approved drugs for the treatment or
prevention of neurodegenerative diseases such as Riluzole, Cognex, Aricept, Sinmet,
Sinmet CR, Permax, Parlodel, Elepryl, Symmetrel, Artane); glutamate excitotoxicity
inhibitors (such as glutamate uptake and biosynthesis modulation with compounds like
gabapentin and Riluzole); growth factors like CNTF, BDNF, IGF-1; nitric oxide
10 synthase inhibitors; cyclo-oxygenase inhibitors such as aspirin; ICE inhibitors;
Neuroimmunophilins; N-acetylcysteine and procysteine; antioxidants (such as pyruvate
and lutein), energy enhancers (such as ribose and vincopocetine), vitamins and cofactors
(such as spin traps, CoQ₁₀, carnitine, nicotinamide, Vitamin E or D) lipoic acid,
vinpocetine, other fatty acids (such as docosahexanoic acid (DHA), eicosapentenoic acid
15 (EPA), and gamma linolenic acid (GLA)), various herbal extracts (such as rosemary and
black caraway), and berry oils and meals (such as elderberries, bilberries, english
hawthorn berry, blackberry, blueberry, red and black raspberries). The creatine
compounds could be combined with different neuroprotective agents and administered
together or sequentially.

20 The present invention pertains to methods for modulating a nervous system disease in a subject by administering to the subject a therapeutically effective amount of a combination of creatine, a creatine phosphate or a creatine analog and a neuroprotective agent, such that a nervous system disease is modulated. Additionally, or
25 in place of the neuroprotective agent, a creatine compound can be combined with existing therapeutic drugs for neurodegenerative diseases.

Creatine compounds which are particularly effective for this purpose include creatine, creatine phosphate, and analogs thereof which are described in detail below.

The term "creatine compounds" will be used herein to include creatine, creatine phosphate, and compounds which are structurally similar to creatine or creatine phosphate, analogs of creatine and creatine phosphate, and combinations thereof. The term "creatine compounds" also includes compounds which "mimic" the activity of creatine, creatine phosphate or creatine analogs, i.e., compounds which inhibit or modulate the creatine kinase system. The term creatine compound is also intended to include pharmaceutically acceptable or physiologically acceptable salts of the compounds. Creatine compounds have previously been described in copending application Ser. No. 07/061,677 entitled Methods of Treating Body Parts Susceptible to Ischemia Using Creatine Analogs, filed May 14, 1993; copending application Ser. No. 08/009,638 entitled Creatine Phosphate, Creatine Phosphate Analogs and Uses Therefor, filed on Jan. 27, 1993; copending application Ser. No. 07/812,561 entitled

5 Creatine Analogs Having Antiviral Activity, filed Dec. 20, 1991; and copending
application Ser. No. 07/610,418 entitled Method of Inhibiting transformation of Cells
in Which Purine Metabolic Enzyme Activity is Elevated, filed Nov. 7, 1990. The
entire contents of each of the copending applications are herein expressly
10 incorporated by reference, along with their published foreign counterparts; and all of
the creatine compounds along with their methods of synthesis and discussed in the
aforementioned applications are intended to be part of this invention unless
specifically stated otherwise.

15 The term "mimics" is intended to include compounds which may not be
structurally similar to creatine but mimic the therapeutic activity of creatine, creatine
phosphate or structurally similar compounds. The term "inhibitors of creatine kinase
system" are compounds which inhibit the activity of the creatine kinase enzyme,
molecules that inhibit the creatine transporter or molecules that inhibit the binding of
20 the enzyme to other structural proteins, enzymes or lipids. The term "modulators of
the creatine kinase system" are compounds which modulate the activity of the
enzyme, or the activity of the transporter of creatine or the ability of other proteins or
enzymes or lipids to interact with the system. The term "creatine analog" is intended
to include compounds which are structurally similar to creatine or creatine
phosphate, compounds which are art-recognized as being analogs of creatine or
25 creatine phosphate, and/or compounds which share the same or similar function as
creatine or creatine phosphate.

30 The language "modulating a nervous system disease" or "modulating a
disease of the nervous system" is intended to include prevention of the disease,
amelioration and/or arrest of a preexisting disease, or the elimination of a preexisting
disease. The combinations of creatine analogs and neuroprotective agents described
herein have both curative and prophylactic effects on disease development and
progression.

35 The language "therapeutically effective amount" is intended to include the
amount of a combination of a creatine compound and neuroprotective agent
sufficient to prevent onset of diseases of the nervous system or significantly reduce
progression of such diseases in the subject being treated. A therapeutically effective
amount can be determined on an individual basis and will be based, at least in part,
40 on consideration of the severity of the symptoms to be treated and the activity of the
specific analog selected if an analog is being used. Further, the effective amounts of

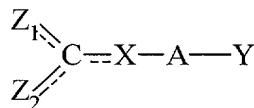
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5 the creatine compound(s) and neuroprotective agent(s) may vary according to the age, sex and weight of the subject being treated. Thus, a therapeutically effective amount of the combinations can be determined by one of ordinary skill in the art employing such factors as described above using no more than routine experimentation in clinical management.

10

The present invention also pertains to methods for modulating a nervous system disease in a subject by administering to the subject a therapeutically effective amount of a combination of a creatine compound and a neuroprotective agent such that a nervous system disease is modulated. The creatine compound has the formula:

15



and pharmaceutically acceptable salts thereof, wherein:

20

a) Y is selected from the group consisting of: -CO₂H, -NHOH, -NO₂, -SO₃H, -C(=O)NHSO₂J and -P(=O)(OH)(OJ), wherein J is selected from the group consisting of: hydrogen, C₁-C₆ straight chain alkyl, C₃-C₆ branched alkyl, C₂-C₆ alkenyl, C₃-C₆ branched alkenyl, and aryl;

25

b) A is selected from the group consisting of: C, CH, C₁-C₅alkyl, C₂-C₅alkenyl, C₂-C₅alkynyl, and C₁-C₅ alkoyl chain, each having 0-2 substituents which are selected independently from the group consisting of:

30

1) K, where K is selected from the group consisting of: C₁-C₆ straight alkyl, C₂-C₆ straight alkenyl, C₁-C₆ straight alkoyl, C₃-C₆ branched alkyl, C₃-C₆ branched alkenyl, and C₄-C₆ branched alkoyl, K having 0-2 substituents independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

35

2) an aryl group selected from the group consisting of: a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents independently selected from the group consisting of: -CH₂L and -COCH₂L where L is independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy; and

5 3) -NH-M, wherein M is selected from the group consisting of:
hydrogen, C₁-C₄ alkyl, C₂-C₄ alkenyl, C₁-C₄ alkoyl, C₃-C₄ branched alkyl, C₃-C₄
branched alkenyl, and C₄ branched alkoyl;

c) X is selected from the group consisting of NR₁, CHR₁, CR₁, O and S,
10 wherein R₁ is selected from the group consisting of:

1) hydrogen;

2) K where K is selected from the group consisting of: C₁-C₆ straight alkyl, C₂-C₆ straight alkenyl, C₁-C₆ straight alkoyl, C₃-C₆ branched alkyl, C₃-C₆ branched alkenyl, and C₄-C₆ branched alkoyl, K having 0-2 substituents independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

20 3) an aryl group selected from the group consisting of a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents independently selected from the group consisting of: $-CH_2L$ and $-COCH_2L$ where L is independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

4) a C₅-C₉ α-amino-ω-methyl-ω-adenosylcarboxylic acid attached
25 via the ω-methyl carbon;

5) a C₅-C₉ a-amino-w-aza-w-methyl-w-adenosylcarboxylic acid attached via the w-methyl carbon; and

30 6) a C₅-C₉ a-amino-w-thia-w-methyl-w-adenosylcarboxylic acid
attached via the w-methyl carbon;

d) Z_1 and Z_2 are chosen independently from the group consisting of: $=0$, $-NHR_2$, $-CH_2R_2$, $-NR_2OH$; wherein Z_1 and Z_2 may not both be $=0$ and wherein R_2 is selected from the group consisting of:

1) hydrogen;

2) K, where K is selected from the group consisting of: C₁-C₆
40 straight alkyl; C₂-C₆ straight alkenyl, C₁-C₆ straight alkoyl, C₃-C₆ branched alkyl,

5 C₃-C₆ branched alkenyl, and C₄-C₆ branched alkoyl, K having 0-2 substituents independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

3) an aryl group selected from the group consisting of a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents
10 independently selected from the group consisting of: -CH₂L and -COCH₂L where L is independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

4) a C₄-C₈ α-amino-carboxylic acid attached via the α-carbon;

15 5) B, wherein B is selected from the group consisting of: -CO₂H, -NHOH, -SO₃H, -NO₂, OP(=O)(OH)(OJ) and -P(=O)(OH)(OJ), wherein J is selected from the group consisting of: hydrogen, C₁-C₆ straight alkyl, C₃-C₆ branched alkyl, C₂-C₆ alkenyl, C₃-C₆ branched alkenyl, and aryl, wherein B is optionally connected to the nitrogen via a linker selected from the group consisting of: C₁-C₂ alkyl, C₂ alkenyl,
20 and C₁-C₂ alkoyl;

6) -D-E, wherein D is selected from the group consisting of: C₁-C₃ straight alkyl, C₃ branched alkyl, C₂-C₃ straight alkenyl, C₃ branched alkenyl, C₁-C₃ straight alkoyl, aryl and aroyl; and E is selected from the group consisting of:
25 -(P(O)₃)_nNMP, where n is 0-2 and NMP is ribonucleotide monophosphate connected via the 5'-phosphate, 3'-phosphate or the aromatic ring of the base; -[P(=O)(OCH₃)(O)]_m-Q, where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base; -[P(=O)(OH)(CH₂)]_m-Q, where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base; and an aryl group containing 0-3
30 substituents chosen independently from the group consisting of: Cl, Br, epoxy, acetoxy, -OG, -C(=O)G, and -CO₂G, where G is independently selected from the group consisting of: C₁-C₆ straight alkyl, C₂-C₆ straight alkenyl, C₁-C₆ straight alkoyl, C₃-C₆ branched alkyl, C₃-C₆ branched alkenyl, C₄-C₆ branched alkoyl, wherein E may be attached to any point to D, and if D is alkyl or alkenyl, D may be connected at either
35 or both ends by an amide linkage; and

7) -E, wherein E is selected from the group consisting of -
(P(O)₃)_nNMP, where n is 0-2 and NMP is a ribonucleotide monophosphate connected via the 5'-phosphate, 3'-phosphate or the aromatic ring of the base; -[P(=O)(OCH₃)(O)]_m-Q,
40 where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base; -[P(=O)(OH)(CH₂)]_m-Q, where m is 0-3 and Q is a ribonucleoside connected

5 via the ribose or the aromatic ring of the base; and an aryl group containing 0-3
substituents chose independently from the group consisting of: C₁, Br, epoxy, acetoxy,
-OG, -C(=O)G, and -CO=G, where G is independently selected from the group
consisting of: C₁-C₆ straight alkyl, C₂-C₆ straight alkenyl, C₁-C₆ straight alkoyl, C₃-C₆
branched alkyl, C₃-C₆ branched alkenyl, C₄-C₆ branched alkoyl; and if E is aryl, E may
10 be connected by an amide linkage;

e) if R₁ and at least one R₂ group are present, R₁ may be connected by a single or double bond to an R₇ group to form a cycle of 5 to 7 members;

15 f) if two R₂ groups are present, they may be connected by a single or a double bond to form a cycle of 4 to 7 members; and

g) if R_1 is present and Z_1 or Z_2 is selected from the group consisting of -
NHR₂, -CH₂R₂ and -NR₂OH, then R_1 may be connected by a single or double bond to
20 the carbon or nitrogen of either Z_1 or Z_2 to form a cycle of 4 to 7 members.

Additionally, or in place of the neuroprotective agent, a creatine compound can be combined with existing therapeutic drugs for neurodegenerative diseases.

The term "neuroprotective agent" is intended to include those compositions which prevent depletion of ATP prevent glutamate excitotoxicity or prevent production of free radicals or other agents which interfere with, destroy, or diminish nervous system activity. Representative neuroprotective agents include approved drugs for the treatment or prevention of neurodegenerative diseases such as Riluzole, Cognex, Aricept, Sinmet, Sinmet CR, Permax, Parlodel, Elepryl, Symmetrel, Artane); glutamate excitotoxicity inhibitors (such as glutamate uptake and biosynthesis modulation with compounds like gabapentin and Riluzole); growth factors like CNTF, BDNF, IGF-1; nitric oxide synthase inhibitors; cyclo-oxygenase inhibitors such as aspirin; ICE inhibitors; Neuroimmunophilins; N-acetylcysteine and procysteine; antioxidants (such as pyruvate and lutein), energy enhancers (such as ribose and vincopocetine), vitamins and cofactors (such as spin traps, CoQ₁₀, carnitine, nicotinamide, Vitamin E or D) lipoic acid, vinpocetine, other fatty acids (such as docosahexanoic acid (DHA), eicosapentenoic acid (EPA), and gamma linolenic acid (GLA)), various herbal extracts (such as rosemary and black caraway), and berry oils and meals (such as bilberry, elderberry, english hawthorn berry, blackberry, blueberry, red and black raspberries).

5 The present invention further pertains to pharmaceutical compositions for
modulating a nervous system disease in a subject. The pharmaceutical compositions
include an effective amount, e.g. synergistically effective amount, of a combination of a
creatine compound having the formula described above, a neuroprotective agent and a
pharmaceutically acceptable carrier. In preferred embodiments, the creatine compound
10 is creatine, creatine phosphate, cyclocreatine or cyclocreatine phosphate beta guanidino
propionic acid.

 The present invention also pertains to packaged nervous system disease
modulators which include a creatine compound having the formula described above
15 and at least one neuroprotective agent. Additionally, or in place of the
neuroprotective agent, a creatine compound can be combined with existing
therapeutic drugs for neurodegenerative diseases.

 The language "pharmaceutically acceptable carrier" is intended to include
20 substances capable of being coadministered with the creatine compound(s) and
neuroprotective agent(s) and which allows the active ingredients to perform their
intended function of preventing, ameliorating, arresting, or eliminating a disease(s) of
the nervous system. Examples of such carriers include agents to enhance creatine
compound uptake such as sugars, solvents, dispersion media, adjuvants, delay agents
25 and the like. The use of such media and agents for pharmaceutically active
substances is well known in the art. Any conventional media and agent compatible
with the creatine compound may be used within this invention.

 The term "pharmaceutically acceptable salt" is intended to include art-
30 recognized pharmaceutically acceptable salts. Typically these salts are capable of
being hydrolyzed under physiological conditions. Examples of such salts include
sodium, potassium and hemisulfate. The term further is intended to include lower
hydrocarbon groups capable of being hydrolyzed under physiological conditions, i.e.
groups which esterify the carboxyl moiety, e.g. methyl, ethyl and propyl.

35 The term "subject" is intended to include living organisms susceptible to
having diseases of the nervous system, e.g. mammals. Examples of subjects include
humans, dogs, cats, horses, cows, goats, rats and mice. The term "subject" further is
intended to include transgenic species.

40

5 The present invention pertains to compositions of creatine compounds, including
the formula described above, and neuroprotective agents improved nervous system
function. Preferred creatine compounds include creatine, creatine phosphate,
cyclocreatine or cyclocreatine phosphate beta guanidino propionic acid. Preferred
neuroprotective agents include: approved drugs for the treatment or prevention of
10 neurodegenerative diseases such as Riluzole, Cognex, Aricept, Sinmet, Sinmet CR,
Permax, Parlodel, Elepryl, Symmetrel, Artane); glutamate excitotoxicity inhibitors
(such as glutamate uptake and biosynthesis modulation with compounds like gabapentin
and Riluzole); growth factors like CNTF, BDNF, IGF-1; nitric oxide synthase
inhibitors; cyclo-oxygenase inhibitors such as aspirin; ICE inhibitors;
15 Neuroimmunophilins; N-acetylcysteine and procysteine; antioxidants (such as pyruvate
and lutein), energy enhancers (such as ribose and vincopocetine), vitamins and cofactors
(such as spin traps, CoQ₁₀, carnitine, nicotinamide, Vitamin E or D) lipoic acid,
vinpocetine, other fatty acids (such as docosahexanoic acid (DHA), eicosapentenoic acid
(EPA), and gamma linolenic acid (GLA)), various herbal extracts (such as rosemary and
20 black caraway), and berry oils and meals (such as bilberry, elberberry, english hawthorn
berry, blackberry, blueberry, red and black raspberries).

These compositions of creatine compounds and neuroprotective agents can be
used as dietary food supplements or medical foods to improve nervous system activities
25 and associated functions. When used as a dietary food supplement or a medical food,
these compositions are included as additives to enhance the ability of the food to protect,
alleviate, and/or enhance the nervous system against nervous system disease states.

The language "diseases of the nervous system" or "nervous system disease" is
30 intended to include diseases of the nervous system whose onset, amelioration, arrest,
or elimination is effectuated by the creatine compounds described herein. Examples
of types of diseases of the nervous system include demyelinating, dysmyelinating
and degenerative diseases. Examples of locations on or within the subject where the
diseases may originate and/or reside include both central and peripheral loci. As the
35 term "disease" is used herein, it is understood to exclude, and only encompass
maladies distinct from, neoplastic pathologies and tumors of the nervous system,
inschemic injury and viral infections of the nervous system. Examples of types of
diseases suitable for treatment with the methods and compounds of the instant
invention are discussed in detail below.

Diseases of the Nervous System

10

15

20

Demyelinating Diseases:

25

Multiple Sclerosis (MS)

40

5 The pathogenesis is due to an autoimmune attack on CNS myelin. The
treatments available are symptomatic treating spasticity, fatigue, bladder dysfunction,
and spasms. Other treatments are directed towards stopping the immunologic attack
on myelin. These consist of corticosteroids such as prednisone and
10 methylprednisolone, general immunosuppressants such as cyclophosphamide and
azathioprine, and immunomodulating agents such as beta-interferon. No treatments
are available to preserve myelin or make it resistant to attacks.

Acute Disseminated Encephalomyelitis

15 Acute Disseminated Encephalomyelitis usually occurs following a viral
infection and is thought to be due to an autoimmune reaction against CNS myelin,
resulting in paralysis, lethargy, and coma. It differs from MS by being a monophasic
disease whereas MS is characterized by recurrence and chronicity. Treatment
consists of administration of steroids.

20 Acute Necrotizing Hemorrhagic Leukoencephalitis

This is a rare disease that is generally fatal. It is also thought to be mediated
by autoimmune attack on CNS myelin that is triggered by a viral infection.
Neurologic symptoms develop abruptly with headache, paralysis and coma. Death
usually follows within several days. Treatment is supportive.

25

Leukodystrophies

These are diseases of the white matter resulting from an error in the myelin
metabolism that leads to impaired myelin formation. They are thought of as
dysmyelinating diseases, and can become manifest at an early age.

30

Metachromatic Leukodystrophy: an autosomal recessive (inherited) disorder
due to deficiency of the enzyme arylsulfatase A leading to accumulation of lipids.
There is demyelination in the CNS and peripheral nervous system leading to
progressive weakness and spasticity.

35

Krabbe's disease: Also inherited as autosomal recessive and due to deficiency
of another enzyme: galactocerebroside beta-galactosidase.

40 Adrenoleukodystrophy and adrenomyeloneuropathy: affect the adrenal gland
in addition to the nervous system.

5 No treatment is available to any of the leukodystrophies except for supportive treatment

Degenerative Diseases:

10 There is no good etiology or pathophysiology known for these diseases, and no compelling reason to assume that they all have a similar etiology. Diseases under this category have general similarities. They are diseases of neurons that tend to result in selective impairment, affecting one or more functional systems of neurons while leaving others intact.

15 Parkinson's Disease:

 Parkinson's disease is due to loss of dopaminergic neurones in the substantia nigra of the brain. It is manifested by slowed voluntary movements, rigidity, expressionless face and stooped posture. Several drugs are available to increase dopaminergic function such as levodopa, carbidopa, bromocriptine, pergolide, or
20 decrease cholinergic function such as benztropine, and amantadine. Selegiline is a new treatment designed to protect the remaining dopaminergic neurons.

Spinocerebellar Degenerations

25 This is a group of degenerative diseases that affects in varying degrees the basal ganglia, brain stem, cerebellum, spinal cord, and peripheral nerves. Patients present symptoms of Parkinsonism, ataxia, spasticity, and motor and sensory deficits reflecting damage to different anatomic areas and/or neuronal systems in the CNS.

Degenerative Disease Affecting Motor Neurons

30 Included in this category are diseases such as amyotrophic lateral sclerosis (ALS), and spinal muscular atrophy. They are characterized by degeneration of motor neurones in the CNS leading to progressive weakness, muscle atrophy, and death caused by respiratory failure. Treatments are only symptomatic, there are no available treatments to slow down or stop the disease.

35

Alzheimer Disease (AD):

 This disease is characterized clinically by slow erosion of mental function, culminating in profound dementia. The diagnostic pathologic hallmark of AD is the presence of large numbers of senile plaques and neurofibrillary tangles in the brain
40 especially in neocortex and hippocampus. Loss of specific neuron populations in these brain regions and in several subcortical nuclei correlates with depletion in

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5 certain neurotransmitters including acetylcholine. The etiology of AD is still
unknown. To date a lot of research has focused on the composition and genesis of
the B/A4 amyloid component of senile plaques. Alzheimer's disease is characterized
clinically by the slow erosion of intellectual function with the development of
profound dementia. There are no treatments that slow the progression.

10

Huntington Disease (HD):

HD is an autosomal dominant disorder of midlife onset, characterized
clinically by movement disorder, personality changes, and dementia often leading to
death in 15-20 years. The neuropathologic changes in the brain are centered in the
15 basal ganglia. Loss of a class of projection neurons, called "spiny cells" because of
their prominent dendritic spinous processes, is typical. This class of cells contains
gamma-aminobutyric acid (GABA), substance P, and opioid peptides. Linkage
studies have localized the gene for HD to the most distal band of the short arm of
chromosome 4. No treatments are available that have been shown to retard
20 progression of the disease. Experimental studies showing a similarity between
neurons that are susceptible to N-methyl d-aspartate (NMDA) agonists and those that
disappear in HD has led to encouraging speculation that NMDA antagonists might
prove beneficial. Some recent studies suggest that a defect in brain energy
metabolism might occur in HD and enhance neuronal vulnerability to excitotoxic
25 stress.

Mitochondrial Encephalomyopathies:

Mitochondrial encephalomyopathies are a heterogenous group of disorders
affecting mitochondrial metabolism. These deficits could involve substrate transport,
30 substrate utilization, defects of the Krebs Cycle, defects of the respiratory chain, and
defects of oxidation/phosphorylation coupling. Pure myopathies vary considerably
with respect to age at onset, course (rapidly progressive, static, or even reversible),
and distribution of weakness (generalized with respiratory failure, proximal more
than distal facioscapulohumeral, orbicularis and extraocular muscles with ptosis and
35 progressive external ophthalmoplegia). Patients with mitochondrial myopathies
complain of exercise intolerance and premature fatigue.

Peripheral Nervous System Disorders

The peripheral nervous system (PNS) consists of the motor and sensory
40 components of the cranial and spinal nerves, the autonomic nervous system with its
sympathetic and parasympathetic divisions, and the peripheral ganglia. It is the

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5 conduit for sensory information to the CNS and effector signals to the peripheral
organs such as muscle. Contrary to the brain, which has no ability to regenerate, the
pathologic reactions of the PNS include both degeneration and regeneration. There
are three basic pathological processes: Wallerian degeneration, axonal degeneration
and segmental demyelination that could take place.

10

Some of the neuropathic syndromes include:

Acute ascending motor paralysis with variable sensory disturbance; examples being acute demyelinating neuropathies, infectious mononucleosis with polyneuritis, hepatitis and polyneuritis, toxic polyneuropathies.

Subacute sensorimotor polyneuropathy; examples of acquired axonal neuropathics include paraproteinemias, uremia diabetes, amyloidosis, connective tissue diseases and leprosy. Examples of inherited diseases include mostly chronic demyelination with hypertrophic changes, such as peroneal muscular atrophy, hypertrophic polyneuropathy and Refsum's diseases.

Chronic relapsing polyneuropathy; such as idiopathic polyneuritis porphyria,
Beriberi and intoxications.

25

Mono or multiple neuropathy; such as pressure palsies, traumatic palsies, serum neuritis, zoster and leprosy.

Aging:

During the process of aging increased oxidative damage and impaired mitochondrial functions contribute to neuronal cell death. Mitochondria are deeply involved in the production of reactive oxygen species and are themselves highly susceptible to oxidative stress which results in apoptotic cell death. Accumulation of mutations in the mitochondrial DNA seems to contribute to the process of aging as evident by respiratory chain function defects and mutations in mtDNA with aging.

The methods and compounds of this invention can also be used to treat neuromuscular disorders and epilepsy.

5

Creatine Compounds Useful For Treating Nervous System Diseases

10 Creatine compounds useful in the present invention include compounds which modulate one or more of the structural or functional components of the creatine kinase/phosphocreatine system. Compounds which are effective for this purpose include creatine, creatine phosphate and analogs thereof, compounds which mimic their activity, and salts of these compounds as defined above. Exemplary creatine compounds are described below.

15 Creatine (also known as N-(aminoiminomethyl)-N-methylglycine; methylglycosamine or N-methyl-guanido acetic acid) is a well-known substance. (See, The Merck Index, Eleventh Edition, No. 2570 (1989).

20 Creatine is phosphorylated chemically or enzymatically by creatine kinase to generate creatine phosphate, which also is well-known (see, The Merck Index, No. 7315). Both creatine and creatine phosphate (phosphocreatine) can be extracted from animal tissue or synthesized chemically. Both are commercially available.

25 Cyclocreatine is an essentially planar cyclic analog of creatine. Although cyclocreatine is structurally similar to creatine, the two compounds are distinguishable both kinetically and thermodynamically. Cyclocreatine is phosphorylated efficiently by creatine kinase in the forward reaction both *in vitro* and *in vivo* (Rowley, G.L., *J. Am. Chem. Soc.* 93: 5542-5551 (1971); McLaughlin, A.C. et. al., *J. Biol. Chem.* 247:4382-4388 (1972)).

30

The phosphorylated compound phosphocyclocreatine is structurally similar to phosphocreatine; however, the phosphorous-nitrogen (P-N) bond of cyclocreatine phosphate is more stable than that of phosphocreatine. LoPresti, P. and M. Cohn, *Biochem. Biophys. Acta* 998:317-320 (1989); Annesley, T. M. and J. B. Walker, *J. Biol. Chem.* 253:8120-8125 (1978); Annesley, T.M. and J.B. Walker, *Biochem. Biophys. Res. Commun.* 74:185-190 (1977).

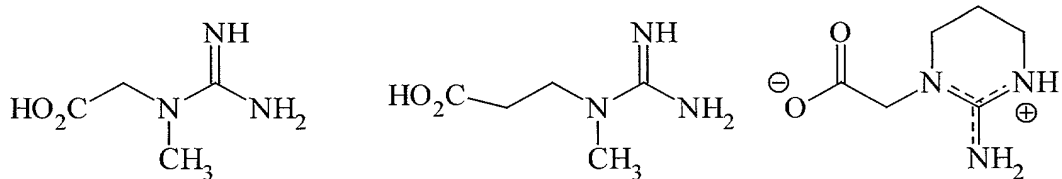
40 Creatine analogs and other agents which act to interfere with the activity of creatine biosynthetic enzymes or with the creatine transporter are useful in the present method of treating nervous system diseases. In the nervous system, there are many possible intracellular, as well as extracellular, sites for the action of compounds that inhibit, increase, or otherwise modify, energy generation through brain creatine

- 5 kinase and/or other enzymes which are associated with it. Thus the effects of such
compounds can be direct or indirect, operating by mechanisms including, but not
limited to, influencing the uptake or biosynthesis of creatine, the function of the
creatine phosphate shuttle, inhibiting the enzyme activity, or the activity of
associated enzymes, or altering the levels of substrates or products of a reaction to
10 alter the velocity of the reaction.

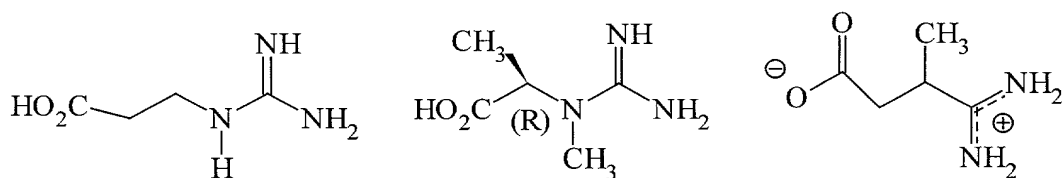
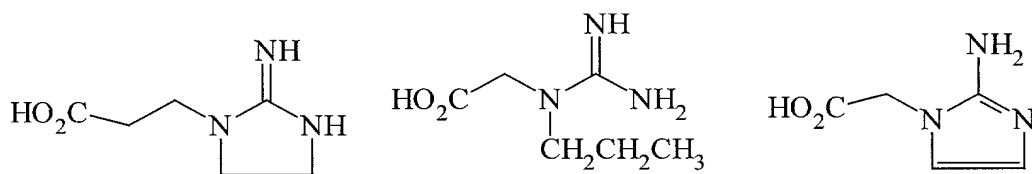
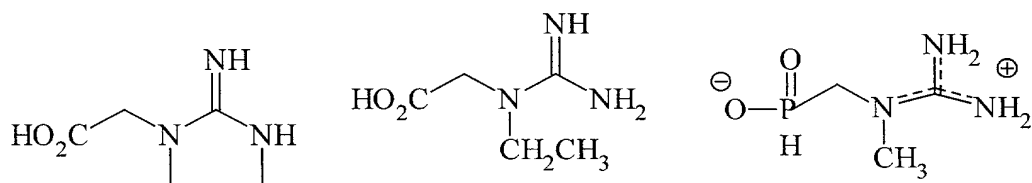
Substances known or believed to modify energy production through the creatine kinase/phosphocreatine system which can be used in the present method are described below. Exemplary compounds are shown in Tables 1 and 2.

5

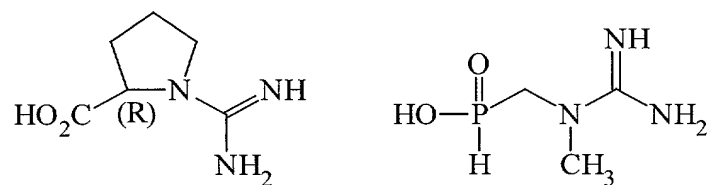
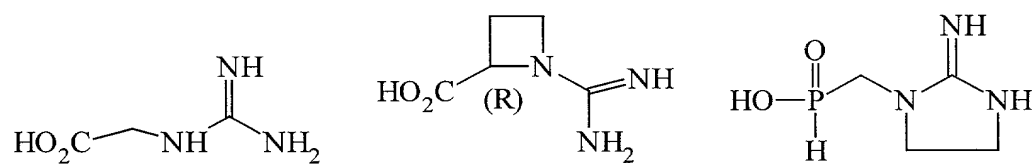
TABLE 1
CREATINE ANALOGS



10

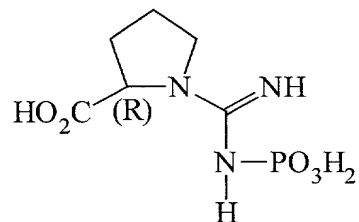
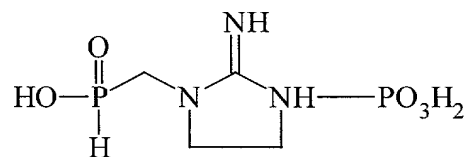
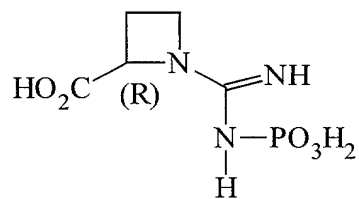
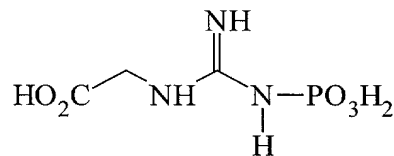
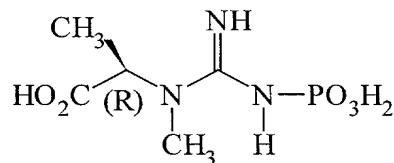
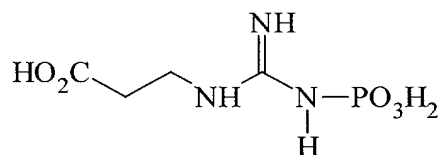
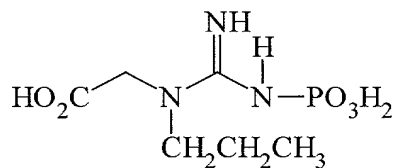
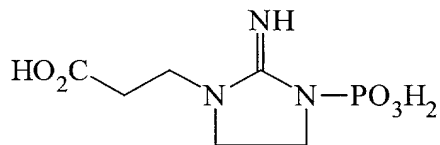
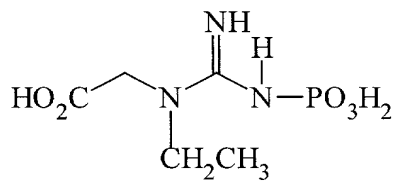
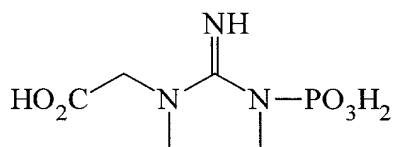
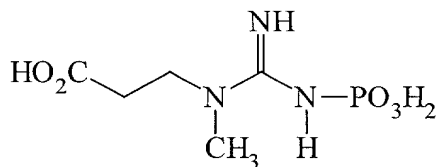
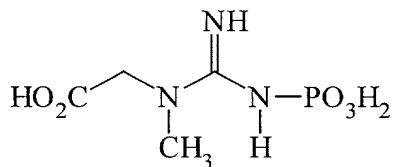


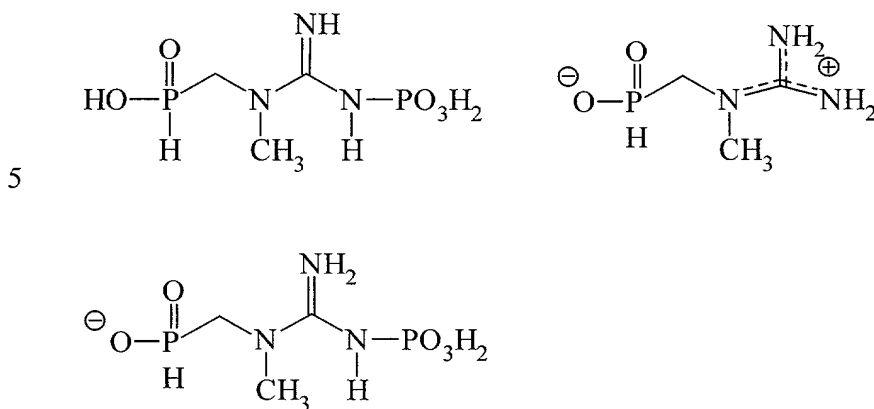
15



5

TABLE 2
CREATINE ANALOGS





It will be possible to modify the substances described below to produce analogs which have enhanced characteristics, such as greater specificity for the enzyme, enhanced stability, enhanced uptake into cells, or better binding activity.

Compounds which modify the structure or function of the creatine kinase/creatine phosphate system directly or indirectly are useful in preventing and/or treating diseases of the nervous system characterized by up regulation or down regulation of the enzyme system.

In diseases where the creatine kinase/creatine phosphate system is down regulated, for example, uncontrolled firing of neurons, molecules useful for treating these diseases include those that will up regulate the activity, or could support energy (ATP) production for a longer period of time. Examples include creatine phosphate and related molecules that form stable phosphagens which support ATP production over a long period of time.

In diseases where the creatine kinase/creatine phosphate system is up regulated, the molecules that are useful include those that will down regulate the activity and/or inhibit energy production (ATP).

Molecules that regulate the transporter of creatine, or the association of creatine kinase with other protein or lipid molecules in the membrane, the substrates concentration creatine and creatine phosphate also are useful in preventing and/or treating diseases of the nervous system.

5 Compounds which are useful in the present invention can be inhibitors, substrates or substrate analogs, of creatine kinase, which when present, could modify energy generation or high energy phosphoryl transfer through the creatine kinase/phosphocreatine system. In addition, modulators of the enzymes that work in conjunction with creatine kinase now can be designed and used, individually, in
10 combination or in addition to other drugs, to make control of the effect on brain creatine kinase tighter.

 The pathways of biosynthesis and metabolism of creatine and creatine phosphate can be targeted in selecting and designing compounds which modify
15 energy production or high energy phosphoryl transfer through the creatine kinase system. Compounds targeted to specific steps may rely on structural analogies with either creatine or its precursors. Novel creatine analogs differing from creatine by substitution, chain extension, and/or cyclization may be designed. The substrates of multisubstrate enzymes may be covalently linked, or analogs which mimic portions
20 of the different substrates may be designed. Non-hydrolyzable phosphorylated analogs can also be designed to mimic creatine phosphate without sustaining ATP production.

 A number of creatine and creatine phosphate analogs have been previously
25 described in the literature or can be readily synthesized. Examples are these shown in Table I and Table 2. Some of them are slow substrates for creatine kinase.

 Tables 1 and 2 illustrate the structures of creatine, cyclocreatine (1-carboxymethyl-2-iminoimidazolidine), N-phosphorocreatine (N-phosphoryl
30 creatine), cyclocreatine phosphate (3-phosphoryl-1-carboxymethyl-2-iminoimidazolidine) and other compounds. In addition, 1-carboxymethyl-2-aminoimidazole, 1-carboxymethyl-2, 2-iminomethylimidazolidine, 1-carboxyethyl-2-iminoimidazolidine, N-ethyl-N-amidinoglycine and b-guanidinopropionic acid are believed to be effective.

35 Cyclocreatine (1-carboxymethyl-2-iminoimidazolidine) is an example of a class of substrate analogs of creatine kinase, which can be phosphorylated by creatine kinase and which are believed to be active.

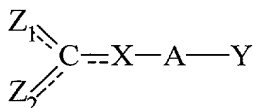
40 A class of creatine kinase targeted compounds are bi-substrate analogs comprising an adenosine-like moiety linked via a modifiable bridge to a creatine link

5 moiety (i.e., creatine or a creatine analog). Such compounds are expected to bind
with greater affinity than the sum of the binding interaction of each individual
substrate (e.g., creatine and ATP). The modifiable bridge linking an adenosine like
moiety at the 5' carbon to a creatine like moiety can be a carbonyl group, alkyl (a
10 branched or straight chain hydrocarbon group having one or more carbon atoms), or
substituted alkyl group (an alkyl group bearing one or more functionalities, including
but not limited to unsaturation, heteroatom substituents, carboxylic and inorganic
acid derivatives, and electrophilic moieties).

15 Another class of potential compounds for treating nervous system disorders is
designed to inhibit (reversibly or irreversibly) creatine kinase. The analogs of
creatine in this class can bind irreversibly to the active site of the enzyme. Two such
affinity reagents that have previously been shown to completely and irreversibly
inactivate creatine kinase are epoxycreatine Marietta, M.A. and G.L. Kenyon *J. Biol*
20 *Chem.* 254: 1879-1886 (1979)) and isoepoxycreatine. There are several approaches
to enhancing the specificity and hence, the efficacy of active site-targeted irreversible
inhibitors of creatine kinase, incorporating an electrophilic moiety. The effective
concentration of a compound required for inhibition can be lowered by increasing
favorable and decreasing unfavorable binding contacts in the creatine analog.

25 N-phosphorocreatine analogs also can be designed which bear non-
transferable moieties which mimic the N-phosphoryl group. These cannot sustain
ATP production.

30 Some currently preferred creatine compounds of this invention are those
encompassed by the general formula I:



and pharmaceutically acceptable salts thereof, wherein:

35 a) Y is selected from the group consisting of: -CO₂H-NHOH, -N₃, -SO₃H,
-C(=O)NHSO₂J and -P(=O)(OH)(OJ), wherein J is selected from the group
consisting of: hydrogen, C₁-C₆ straight chain alkyl, C₃-C₆ branched alkyl,
C₂-C₆ alkenyl, C₃-C₆ branched alkenyl, and aryl;

5 b) A is selected from the group consisting of: C, CH, C₁-C₅alkyl, C₂-C₅alkenyl, C₂-C₅alkynyl, and C₁-C₅alkoyl chain, each having 0-2 substituents which are selected independently from the group consisting of:

10 1) K, where K is selected from the group consisting of: C₁-C₆ straight alkyl, C₂-C₆ straight alkenyl, C₁-C₆ straight alkoyl, C₃-C₆ branched alkyl, C₃-C₆ branched alkenyl, and C₄-C₆ branched alkoyl, K having 0-2 substituents independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

15 2) an aryl group selected from the group consisting of: a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents independently selected from the group consisting of: -CH₂L and -COCH₂L where L is independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy; and

20 3) -NH-M, wherein M is selected from the group consisting of: hydrogen, C₁-C₄ alkyl, C₂-C₄ alkenyl, C₁-C₄ alkoyl, C₃-C₄ branched alkyl, C₃-C₄ branched alkenyl, and C₄ branched alkoyl;

25 c) X is selected from the group consisting of NR₁, wherein R₁ is selected from the group consisting of:

1) hydrogen;

30 2) K where K is selected from the group consisting of: C₁-C₆ straight alkyl, C₂-C₆ straight alkenyl, C₁-C₆ straight alkoyl, C₃-C₆ branched alkyl, C₃-C₆ branched alkenyl, and C₄-C₆ branched alkoyl, K having 0-2 substituents independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

35 3) an aryl group selected from the group consisting of a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents independently selected from the group consisting of: -CH₂L and -COCH₂L where L is independently selected from the group consisting of:
40 bromo, chloro, epoxy and acetoxy;

5 4) a Cs-Cg a-amino-w-methyl-w-adenosylcarboxylic acid attached via
the w-methyl carbon;

5) 2 Cs-Cg a-amino-w-aza-w-methyl-w-adenosylcarboxylic acid attached via the w-methyl carbon; and

6) a Cs-Cg a-amino-w-thia-w-methyl-w-adenosylcarboxylic acid
attached via the w-methyl carbon;

15 d) Z_1 and Z_2 are chosen independently from the group consisting of: =O, -NHR₂, -CH₂R₂, -NR₂OH; wherein Z_1 and Z_2 may not both be =O and wherein R₂ is selected from the group consisting of:

1) hydrogen;

20 2) K, where K is selected from the group consisting of: C₁-C₆ straight alkyl; C₂-C₆ straight alkenyl, C₁-C₆ straight alkoyl, C₃-C₆ branched alkyl, C₃-C₆ branched alkenyl, and C₄-C₆ branched alkoyl, K having 0-2 substituents independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

3) an aryl group selected from the group consisting of a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents independently selected from the group consisting of: $-CH_2L$ and $-COCH_2L$ where L is independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

4) 2 C₄-C₈ α-amino-carboxylic acid attached via the ω-carbon;

35 5) B, wherein B is selected from the group consisting of: -CO₂H-NHOH, -SO₃H, -NO₂, OP(=O)(OH)(OJ) and -P(=O)(OH)(OJ), wherein J is selected from the group consisting of: hydrogen, C₁-C₆ straight alkyl, C₃-C₆ branched alkyl, C₂-C₆ alkenyl, C₃-C₆ branched alkenyl, and aryl, wherein B is optionally connected to the nitrogen via a linker selected from the group consisting of: C₁-C₂ alkyl, C₂ alkenyl, and C₁-C₂ alkoyl;

5 6) -D-E, wherein D is selected from the group consisting of: C₁-C₃
straight alkyl, C₃ branched alkyl, C₂-C₃ straight alkenyl, C₃ branched
alkenyl, C₁ -C₃ straight alkoyl, aryl and aroyl; and E is selected from the
10 group consisting of: -(P(O)₃)_nNMP, where n is 0-2 and NMP is ribonucleotide
monophosphate connected via the 5'-phosphate, 3'-phosphate or the aromatic
ring of the base; -[P(=O)(OCH₃)(O)]_m-Q, where m is 0-3 and Q is a
ribonucleoside connected via the ribose or the aromatic ring of the base;
-[P(=O)(OH)(CH₂)]_m-Q, where m is 0-3 and Q is a ribonucleoside connected
15 via the ribose or the aromatic ring of the base; and an aryl group containing 0-
3 substituents chosen independently from the group consisting of: Cl, Br,
epoxy, acetoxy, -OG, -C(=O)G, and -CO₂G, where G is independently
selected from the group consisting of: C₁ -C₆ straight alkyl, C₂-C₆ straight
alkenyl, C₁-C₆ straight alkoyl, C₃-C₆ branched alkyl, C₃-C₆ branched
alkenyl, C₄-C₆ branched alkoyl, wherein E may be attached to any point to
D, and if D is alkyl or alkenyl, D may be connected at either or both ends by
20 an amide linkage; and

7) -E, wherein E is selected from the group consisting of -
(P(O)₃)_nNMP, where n is 0-2 and NMP is a ribonucleotide monophosphate
connected via the 5'-phosphate, 3'-phosphate or the aromatic ring of the base;
- [P(=O)(OCH₃)(O)]_m-Q, where m is 0-3 and Q is a ribonucleoside connected
via the ribose or the aromatic ring of the base; - [P(=O)(OH)(CH₂)]_m-Q,
where m is 0-3 and Q is a ribonucleoside connected via the ribose or the
aromatic ring of the base; and an aryl group containing 0-3 substituents chosen
independently from the group consisting of: Cl, Br, epoxy, acetoxy, -OG, -
C(=O)G, and -CO₂G, where G is independently selected from the group
consisting of: C₁-C₆ straight alkyl, C₂-C₆ straight alkenyl, C₁-C₆ straight
alkenyl, C₃-C₆ branched alkyl, C₃-C₆ branched alkenyl, C₄-C₆ branched
alkenyl; and if E is aryl, E may be connected by an amide linkage;

35 e) if R₁ and at least one R₂ group are present, R₁ may be connected by a single or double bond to an R₂ group to form a cycle of 5 to 7 members;

f) if two R₂ groups are present, they may be connected by a single or a double bond to form a cycle of 4 to 7 members; and

40

5 g) if R_1 is present and Z_1 or Z_2 is selected from the group consisting of $-NHR_2$, $-CH_2R_2$ and $-NR_2OH$, then R_1 may be connected by a single or double bond to the carbon or nitrogen of either Z_1 or Z_2 to form a cycle of 4 to 7 members.

10 Creatine, creatine phosphate and many creatine analogs, and competitive inhibitors are commercially available. Additionally, analogs of creatine may be synthesized using conventional techniques. For example, creatine can be used as the starting material for synthesizing at least some of the analogs encompassed by formula I. Appropriate synthesis reagents, e.g. alkylating, alkenylating or
15 alkynylating agents may be used to attach the respective groups to target sites. Alternatively, reagents capable of inserting spacer groups may be used to alter the creatine structure. Sites other than the target site are protected using conventional protecting groups while the desired sites are being targeted by synthetic reagents.

20 If the creatine analog contains a ring structure, then the analog may be synthesized in a manner analogous to that described for cyclocreatine (Wang, T., *J. Org. Chem.* 39:3591-3594 (1974)). The various other substituent groups may be introduced before or after the ring is formed.

25 Many creatine analogs have been previously synthesized and described (Rowley et al., *J. Am. Chem. Soc.* 93:5542-5551 (1971); McLaughlin et al., *J. Biol. Chem.* 247:4382-4388 (1972); Lowe et al., *J. Biol. Chem.* 225:3944-3951 (1980); Roberts et al, *J. Biol. Chem.* 260:13502-13508 (1985); Roberts et al., *Arch. Biochem. Biophys.* 220:563-571 (1983), and Griffiths et al, *J. Biol. Chem.* 251:2049-2054
30 (1976)). The contents of all of the forementioned references are expressly incorporated by reference. Further to the forementioned references, Kaddurah-Daouk et al. (W092/08456; WO90/09192; U.S. 5,324,731; U.S. 5,321,030) also provide citations for the synthesis of a plurality of creatine analogs. The contents of all the aforementioned references and patents are incorporated herein by reference.

35 Creatine compounds which currently are available or have been synthesized include, for example, creatine, b-guanidinopropionic acid, guanidinoacetic acid, creatine phosphate disodium salt, cyclocreatine, homocyclocreatine, phosphinic creatine, homocreatine, ethylcreatine, cyclocreatine phosphate dilithium salt and
40 guanidinoacetic acid phosphate disodium salt, among others.

5 Creatine phosphate compounds also can be synthesized chemically or enzymatically. The chemical synthesis is well known. Annesley, T.M. Walker, J.B., *Biochem. Biophys. Res. Commun.*, 74, 185-190(1977); Cramer, F., Scheiffele, E., Vollmar, A., *Chem. Ber.*, (1962), 95, 1670-1682.

Salts of the products may be exchanged to other salts using standard protocols. The enzymatic synthesis utilizes the creatine kinase enzyme, which is commercially available, to phosphorylate the creatine compounds. ATP is required by creatine kinase for phosphorylation, hence it needs to be continuously replenished to drive the reaction forward. It is necessary to couple the creatine kinase reaction to another reaction that generates ATP to drive it forward. The purity of the resulting compounds can be confirmed using known analytical techniques including ^1H NMR, ^{13}C NMR Spectra, Thin layer chromatography, HPLC and elemental analysis.

Existing Therapeutic Agents for Neurodegenerative Diseases

20 Therapeutic agents for treatment of neurodegenerative disease which are useful in combination with creatine compounds or creatine compounds and neuroprotective agents are described below.

25 Suitable therapeutic drugs for neurodegenerative diseases include those which
have been approved by, for example, the United States Food and Drug
Administration. Representative drugs useful in treatment of Alzheimer's disease
include Cognex (tacrine) manufactured by Parke Davis which is a first generation
acetylcholinesterase inhibitor and Aricept (donepezil) manufactured by Eisai which is
30 a second generation acetylcholinesterase inhibitor.

Suitable drugs for treatment of Parkinson's Disease include Sinemet (carbidopa/levodopa) and Sinemet CR (carbidopa/levodopa sustained release) manufactured by DuPont Pharma. Levodopa is a metabolic precursor of dopamine that crosses the blood-brain barrier. Carbidopa inhibits conversion of levodopa before it crosses the blood-brain barrier. Permax (pergolide mesylate), manufactured by Athena, and Parlodel (bromocriptine mesylate), manufactured by Novartis, are therapeutic agents for treatment of Parkinson's Disease and are dopamine receptor agonists, often used as an adjunct to Sinemet. Eldepryl (selegiline), manufactured by Somerset, is yet another therapeutic agent for treatment of Parkinson's Disease and inhibits monoamine oxidase and is used as an adjunctive therapy. Symmetrel

5 (amantadine), manufactured by DuPont Pharma, has an unknown mechanism of treatment for Parkinson's Disease. Artane (trihexyphenidyl hydrochloride), manufactured by Lederle, also a suitable therapeutic agent is a muscarinic antagonist and is used as an adjunctive therapy.

10 An example of a therapeutic drug for treatment of ALS is Rilutek (riluzole),
manufactured by Rhone-Poulenc Rorer. Rilutek elicits an inhibitory effect on
glutamate release and has various neuroprotective effects, however, the mode of its
action is unknown.

15 Neuroprotective Agents Useful For Treating Nervous System Diseases

Neuroprotective agents include those compositions which provide neuroprotection, e.g., approved drugs for the treatment or prevention of neurodegenerative diseases such as Riluzole, Cognex, Aricept, Sinmet, Sinmet CR, Permax, Parlodel, Elepryl, Symmetrel, Artane); glutamate excitotoxicity inhibitors (such as glutamate uptake and biosynthesis modulation with compounds like gabapentin and Riluzole); growth factors like CNTF, BDNF, IGF-1; nitric oxide synthase inhibitors; cyclo-oxygenase inhibitors such as aspirin; ICE inhibitors; Neuroimmunophilins; N-acetylcysteine and procysteine; antioxidants, energy enhancers, vitamins and cofactors (such as spin traps, CoQ₁₀, carnitine, nicotinamide, Vitamin E or D) lipoic acid, vinpocetine.

ATP Enhancing Agents Useful for Electron Transport

30 ATP enhancing agents include those compounds which facilitate ATP production. These agents can be critical in the function of electron transport and oxidative phosphorylation and hence ATP production and neuronal cell survival. Examples include:

35 Nicotinamide/Riboflavin:

Riboflavin and nicotinamide are water soluble vitamins and components of coenzymes critical in the function of electron transport and oxidative phosphorylation and hence ATP production. The water soluble vitamins are referred to as the vitamin B complex. Riboflavin (vitamin B2) is a precursor of FAD, and niacin is the precursor of Nicotinamide adenine dinucleotide. Nicotinamide adenine dinucleotide is a major electron acceptor in the oxidation of fuel molecules. The reactive part of NAD⁺ is the

5 nicotinamide ring. In the oxidation of substrates the nicotinamide ring of NAD⁺ accepts
a hydrogen ion and two electrons which are equivalent to a hydride ion. The reduced
form of this carrier is called NADH. The other major electron carrier in the oxidation of
fuel molecules is flavin adenine dinucleotide. FAD like NAD⁺ is a two electron
10 acceptor. Hence the molecules riboflavin and nicotinamide are used as supplements to
drive effectively oxidative phosphorylation and could have significant protective effects
in stress conditions or disease states where energy production and oxidative
phosphorylation are compromised.

Nicotinamide is a B vitamin and is a major component of NAD, and NADP
15 which are critical components in the regulation of electron transport chain and energy
production in the mitochondria. Nicotinamide is the amide of nicotinic acid, is a
crystalline compound of the vitamin B complex, is convertible into nicotine acid in the
body. Nicotinic acid is a group of vitamins of the B complex, central for growth and
health in many animals and important in protein and carbohydrate metabolism. It is
20 found in meat, liver, wheat germ, milk eggs. Also, Niacin is converted to nicotinamide
in the body.

Treatment with nicotinamide in combination with riboflavin (Penn et.al.,
Neurology, 42: 2147-2152, 1992; Bernsen et.al., J. Neurol Sci. 118: 181-187, 1993)
25 result in both biochemical and clinical improvement for patients with mitochondrial
disorders. The combination of nicotinamide and coenzyme Q10 were shown to attenuate
malonate induced energy defects and attenuate the striatal lesions produced by this
compound, i.e., an animal model of Huntington's disease (Beal et.al., Annals of
Neurology, 26: 882-888, 1994). Amounts used were Q10 100-300 mg/kg/day,
30 nicotinamide 500 mg/kg/day, and riboflavin 15 mg/kg/day.

Co-Enzyme Qs (CoQs):

A CoQs is a member of the family of co-enzyme Qs wherein the "s" is the
number of isoprenoid units attached to the quinone ring. CoQ₁₀ is a preferred CoQs of
35 the present invention. CoQ₁₀ is present in virtually all living cells. Although a molecular
structure varies among different types of organisms, the chemical structure of CoQ₁₀ (2,3
dimethoxy-5 methyl-6-decaprenyl benzoquinone) consists of a quinone ring (a molecular
structure of carbon, hydrogen, and oxygen) with a long side chain. The body of the
molecule is always the same but the number of the isoprene units (a 5 carbon chemical
40 unit) attached to the quinone ring varies (human CoQ₁₀ has 10 iso-prenoid units) the side
chain is highly fat soluble which allows coq10 to lodge firmly in membranes inside

5 cells. CoQ₁₀ is a large lipophilic fat soluble nutrient with a mol wt. of 862D. It is very
soluble in chloroform and carbon tetrachloride and insoluble in water. CoQ₁₀ is poorly
absorbed unless it is specially prepared by solubilizing-*emmulsifing* in suitable oils or
emmulsified in a silica base excipient containing a non-ionic surfactant. Multi
10 approaches have been developed to enhance the bio-availability of the compound such as
the use of oily preparations to bypass the liver.

CoQ₁₀ is an essential nutrient that is a co-factor in the mitochondrial electron
transport chain, the biochemical pathway in cellular respiration in which ATP and
metabolic energy is derived, since all cellular functions depend on energy CoQ₁₀ seems
15 to be essential for the health of human tissue. Additionally, CoQ₁₀ similar to Vitamin E,
and K has anti-oxidant activity and scavenges free radical which could add to it's benefit
to minimize injury for example to neuronal cells. Diets could be deficient in providing
sufficient amounts of CoQ₁₀ suggesting that supplementation with this compound could
be of benefit in preserving tissue.

20 CoQ₁₀ was first isolated from beef heart mitochondria by Dr. Frederick Crane in
1957 (Crane et al., *Biochimica et Biophys. Acta*, Vol25:220-221, 1957). In 1958 Prof.
Karl Folkers and co-workers at Merck, Inc. determined the precise chemical structure of
CoQ₁₀: 2,3 dimethoxy-5 methyl-6-decaprenyl benzoquinone, synthesized it and were the
25 first to produce it by fermentation. In the mid 1960's Prof. Yamamura of Japan was the
first to use CoQ₇, a related compound to treat a human disease (congestive heart failure).
Multi clinical trials with CoQ₁₀ followed.

Improved cardiovascular morbidity and mortality have been observed in several
30 clinical studies using CoQ₁₀ as a supplement (Serebruany et al., *J. Cardiovascular
Pharmacology* 28(2):1775-181, 1996). Pretreatment with CoQ₁₀ at 150 mg/day for 7
days suggested some protective benefit for patients undergoing routine vascular
procedures requiring abdominal aortic cross clamping by attenuating the degree of
peroxidative damage (Chello et al., *J. of Cardiovascular Surgery* 37(3):229-235, 1996).
35 Benefit to patients with cardiomyopathy has been suggested with the use of CoQ₁₀ at 100
mg/day for several weeks to years (Manzoli et al, *It. J. Tiss. Reac.* 12(3):173-178, 1990;
Langsjoen. et al., *Int. J. Tiss. Reac.* 12(3):163-168, 1990; Langsjoen. et al., *Am. J.
Cardiol.* (65):521-523, 1990, Langsjoen. et al., *nt. J. Tiss. Reac.* 12(3):169-171, 1990;
Morisco et al., *Clin Invest.* 71:S134-S136, 1993).

40

5 Patients with mitochondrial myopathies placed on CoQ₁₀ supplementation at
100-150 mg/day, for extended periods of time, showed benefit in reversing abnormal
biochemical profiles and muscle function (Nakamura et al., Electromyography and
Clinical Neurophysiology 35(6):365-370, 1995, Gold et al., Eur. Neurology 36(4):191-
196, 1996, Ikerjiri et al. Neurol. 47(2):583-585, 1996). Also patients with mitochondrial
10 myopathies secondary to HIV infection and treatment with AZT might benefit from
CoQ₁₀ supplementation (Dalakas et al., N Eng J Med. 322:1098-1105, 1990). Improved
physical performance in patients with muscle dystrophies was noted upon
supplementation with CoQ₁₀ (Folkers et al., Biochimica et Biophysica Acta-Molecular
Basis of Disease 1271(1):281-286, 1995). The combination of CoQ₁₀ and Nicotinamide
15 blocked striatal lesions produced by the mitochondrial toxin Malonate, an animal model
of Huntington's Disease (Beal et al., Ann. Neuro 36(6):882-888, 1994). The
combination of CoQ₁₀ and Nicotinamide and free radical spin traps protected against
MPTP neurotoxicity, an animal model of Parkinson's Disease (Schulz et al., Exp.
Neurol. 132:279-283, 1995).

20 Free Radical Spin Traps:

Free radicals are formed as food and oxygen are metabolized to produce energy.
These radicals can oxidize and kill cells. Oxidation is a chemical reaction in which a
molecule transfers one or more electrons to another. Stable molecules usually have
25 matched pairs of protons and electrons. In certain reactions, a free radical can be formed
having unpaired electrons. Free radicals tend to be highly reactive, oxidizing agents.
Free radicals can kill cells by damaging cell membranes, cytoskeleton and sensitive
nuclear and mitochondrial DNA. Such intracellular damage can lead to the increase in
calcium, increase in damaging proteases and nucleases and production of interferons,
30 TNF- α and other tissue damaging mediators which lead to disease if overexpressed in
response to oxidative stress. When free radicals interact with non-radicals, the result is
usually a chain reaction. Only when two radicals meet or when antioxidants quench the
reaction is the cascade of damage terminated. The most common reactive oxygen
species (ROS) produced in vivo are hydrogen peroxide H₂O₂, hydroxyl OH, superoxide
35 O₂⁻, perhydroxyl HO₂, nitrogen oxide NO, and alkoxyl RO, and peroxy ROO radicals.

In normal healthy individuals this process is offset by endogenous antioxidants
and cellular repair mechanisms. However as organisms age and in certain diseases, the
process can fall out of balance resulting in debilitating and potentially fatal consequences.
40 Oxidation is important factor in many diseases and disorders such as Parkinson's disease
and Alzheimer's disease, ischemia reperfusion injury associated with stroke and heart

5 attack, and inflammatory conditions such as arthritis and ocular inflammation, AIDS
dementia complex, inflammatory bowel disease and rational neovascularization, and
multiple sclerosis.

10 Oxygen breathing animals have developed powerful antioxidant defense systems
and cellular repair mechanisms to control this damage. Enzymes such as superoxide
dismutase, catalase and glutathione peroxidase and vitamins such as tocopherol, ascorbate
and carotene act to quench radical chain reactions. In general many of these natural
molecules alone do not have great activity when given as supplements because they have
to be produced within the cells to be effective in disease prevention.

15 Spin traps are chemical compounds that can protect cells from damaging effects
of free radicals and hence slow or reverse the oxidation damage associated with these
conditions. Suitable spin traps include PBN, S-PBN, DMPO, TEMPOL, azulenyl based
spin traps, MDL, etc.

20 In an animal model of Parkinson's disease, nicotinamide or the free radical spin
trap N-tert-a-(2-sulphophenyl) nitron were effective in inhibiting moderate dopamine
depletion (Schulz et al., Experimental Neurology 132, 279-283, 1995). In the same
study, Q10 and nicotinamide protected against both mild and moderate depletion of
25 dopamine. These results show that agents which improve mitochondrial energy
production like Q10 and nicotinamide and the free radical scavengers can attenuate mild
to moderate MPTP neurotoxicity.

30 Several free radical spin trap compounds can exert neuroprotective effects
against both excitotoxicity and mitochondrial toxins *in vivo*.

L-Carnitine:

35 Carnitine is an important cofactor for normal cellular metabolism. Optimal
utilization of fuel substrates for ATP generation is dependent on adequate carnitine
stores. Fatty acids are activated on the outer mitochondrial membrane, whereas they are
oxidized in the mitochondrial matrix. Long chain acyl CoA molecules do not readily
traverse the inner mitochondrial membrane, and so a special transport mechanism is
needed. Activated long chain fatty acids are carried across the inner mitochondrial
membrane by carnitine. The acyl group is transferred from the sulfur atom of CoA to
40 the hydroxyl group of carnitine to form acyl carnitine, which diffuses across the inner
mitochondrial membrane. On the matrix side of this membrane the acyl group is

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5 transferred back to CoA; which is thermodynamically feasible because of the O-acyl link in carnitine has high transfer potential. Oxidation of long chain fatty acids provides an excellent source of energy. Deficiencies of carnitine might result in impaired flow of metabolites from one compartment of a cell to another which can result in disease.

10 The supplementation of L-carnitine was shown to have some benefit to chronic hemodialysis patients. patients with cardiovascular diseases, muscle diseases, chronic fatigue, diabetic neuropathies, AIDS patients. Typical doses are 20-30 mg/Kg.

Anti-oxidants:

15 Anti-oxidants include those species of compounds which inhibit or prevent oxidation of tissues, such as vitamin E, alpha-omega fatty acids, BHP, ECGC, etc. such as those known in the art. Other anti-oxidants known in the art include pyruvate and lutein. Anti-oxidants can also be derived from natural sources such as berry meals and oils, e.g., from bilberries, elderberries, blackberries, blueberries, english
20 hawthorn berries, red and black raspberries.

Reactive oxygen species are thought to be involved in a number of types of acute and chronic pathologic conditions in the brain and neural tissue. The metabolic antioxidant alpha-lipoate (thioctic acid, 1, 2-dithiolane-3-pentanoic acid; 1, 2-dithiolane-
25 3 valeric acid; and 6, 8-dithiooctanoic acid) is a low molecular weight substance that is absorbed from the diet and crosses the blood-brain barrier. Alpha-lipoate is taken up and reduced in cells and tissues to dihydrolipoate, which is also exported to the extracellular medium; hence, protection is afforded to both intracellular and extracellular environments. Both alpha-lipoate and especially dihydrolipoate have been shown to be
30 potent antioxidants, to regenerate through redox cycling other antioxidants like vitamin C and vitamin E, and to raise intracellular glutathione levels. Thus, it appears an ideal substance in the treatment of oxidative brain and neural disorders involving free-radical processes. Examination of current research reveals protective effects of these compounds in cerebral ischemia-reperfusion, excitotoxic amino acid brain injury,
35 mitochondrial dysfunction, diabetes and diabetic neuropathy, inborn errors of metabolism, and other causes of acute or chronic damage to brain or neural tissue. Very few neuropharmacological intervention strategies are currently available for the treatment of stroke and numerous other brain disorders involving free radical injury. It is believed that the various metabolic antioxidant properties of alpha-lipoate relate to its
40 possible therapeutic roles in a variety of brain and neuronal tissue pathologies: thiols are central to antioxidant defense in brain and other tissues. The most important thiol

5 antioxidant, glutathione, cannot be directly administered, whereas alpha-lipoic acid can. In vitro, animal, and preliminary human studies indicate that alpha-lipoate may be effective in numerous neurodegenerative disorders.

10 The term "herbal extracts" includes any fraction of an herb or other plant which can be administered to a subject. Preferably, the herbal extract has neuroprotective activity. The term includes any part of the plant (e.g., leaves, seeds, stem, fruit, roots, etc.) which can be administered to a subject. Examples of herbal extracts include rosemary extract and black caraway seeds. Other examples compounds which may be included are extracts from green tea, licorice, tricosanthes, pau d'arco, gotu kola, barley
15 grass, moss, kelp, garlic, astragalus, aloe vera, gingseng, ginko, cayenne, red clover flowers, apple, cherry, apricot, prune, hops, skullcap, valerian root, pomegranate, ashwagandha, borage, Bacopa Monniera, kava, grapes, citrus fruits (e.g., bioflavonoids), carob, ginger, wild milky oat, peppermint, blue-green algae, prickly ash, fo-ti, nutmeg, cardamon, reishi mushrooms, dong quai, kudzu, knotweed, yerba mate, lemon balm,
20 tumeric, basil, vanilla, honey suckle, poria, periwinkle, codonopsis, red peony, lycii berry, chrysanthemum, schizandra, moutan peony, adenophora, os draconis, wheat germ, tang kuai, tremella, eucommia, gnetin, japanese plum, cherokee rose, olive oil, coffee bean, and chamomile.

25 Other neuroprotective agents which may advantageously be added to the compositions include phosphatidyl serine, acetyl-L-carnitine, huperzine A, melatonin, folic acid, choline, thiamin, riboflavin, niacin, biotin, calcium, iron, magnesium, potassium, zinc, iodine, inositol, dibencoside, copper, taurine, pantothenic acid, and phosphatidyl choline.

30

Utility

In the present invention, the combinations of creatine compounds and neuroprotective agents can be administered to an individual (e.g., a mammal), alone or in
35 combination with another compound, for the treatment of diseases of the nervous system. As agents for the treatment of diseases of the nervous system, creatine compounds can interfere with creatine kinase/phosphocreatine functions, thereby preventing, ameliorating, arresting or eliminating direct and/or indirect effects of disease which contribute to symptoms such as paraplegia or memory impairment. Other
40 compounds which can be administered together with the creatine compounds include neurotransmitters, neurotransmitter agonists or antagonists, steroids, corticosteroids

5 (such as prednisone or methyl prednisone) immunomodulating agents (such as beta-interferon), immunosuppressive agents (such as cyclophosphamide or azathioprine), nucleotide analogs, endogenous opioids, or other currently clinically used drugs. When
10 co-administered with creatine compounds, these agents can augment interference with creatine kinase/phosphocreatine cellular functions, thereby preventing, reducing, or eliminating direct and/or indirect effects of disease.

A variety of diseases of the nervous system can be treated with creatine or creatine analogs in combination with neuroprotective agents, including but not limited to those diseases of the nervous system described in detail above. Others include bacterial
15 or fungal infections of the nervous system. These creatine or analog combinations can be used to reduce the severity of a disease, reduce symptoms of primary disease episodes, or prevent or reduce the severity of recurrent active episodes. Creatine, creatine phosphate or analogs such as cyclocreatine and cyclocreatine phosphate can be used to treat progressive diseases. Many creatine analogs can cross the blood-brain
20 barrier. For example, treatment can result in the reduction of tremors in Parkinson's disease, and other clinical symptoms.

Modes of Administration

25 The creatine compound and neuroprotective agent can be administered to the afflicted individual alone or in combination with another creatine analog or other agent. The combinations can be administered as pharmaceutically acceptable salts in a pharmaceutically acceptable carrier, for example. The combinations may be administered to the subject by a variety of routes, including, but not necessarily limited
30 to, oral (dietary), transdermal, or parenteral (e.g., subcutaneous, intramuscular, intravenous injection, bolus or continuous infusion) routes of administration, for example. An effective amount (i.e., one that is sufficient to produce the desired effect in an individual) of a composition comprising a creatine analog and a neuroprotective agent is administered to the individual. The actual amount of drug to be administered will
35 depend on factors such as the size and age of the individual, in addition to the severity of symptoms, other medical conditions and the desired aim of treatment.

Previous studies have described the administration and efficacy of creatine compounds *in vivo*. For example, creatine phosphate has been administered to
40 patients with cardiac diseases by intravenous injection. Up to 8 grams/day were administered with no adverse side effects. The efficacy of selected creatine kinase

5 substrate analogs to sustain ATP levels or delay rigor during ischemic episodes in
muscle has been investigated. On one study, cyclocreatine was fed to mice, rats and
chicks, and appeared to be well-tolerated in these animals. Newly hatched chicks
were fed a diet containing 1% cyclocreatine. In the presence of antibiotics, the
chicks tolerated 1 % cyclocreatine without significant mortality, although the chicks
10 grew more slowly than control chicks (Griffiths, G. R. and J. B. Walker, *J. Biol.*
Chem. 251(7): 2049-2054 (1976)). In another study, mice were fed a diet containing
1% cyclocreatine for 10 days (Annesley, T. M. and J. B. Walker, *J. Biol. Chem.*
253(22): 8120-8125 (1978)). Cyclocreatine has been feed to mice at up to 1% of
their diet for 2 weeks or for over 4 weeks without gross adverse effects. Lillie et al.,
15 *Cancer Res.*, 53: 3172-3178 (1993). Feeding animals cyclocreatine (e.g., 1% dietary)
has been shown to lead to accumulation of cyclocreatine in different organs in mM
concentrations. For example, cyclocreatine was reported to be taken up by muscle,
heart and brain in rats receiving dietary 1% cyclocreatine. Griffiths, G. R. and J. B.
Walker, *J. Biol. Chem.* 251(7): 2049-2054 (1976). As shown previously, antiviral
20 activity of cyclocreatine is observed on administering 1% dietary cyclocreatine.
Many of the above-referenced studies show that creatine analogs are been shown to
be capable of crossing the blood-brain barrier.

The creatine compound and neuroprotective agent combination can be
25 formulated according to the selected route of administration (e.g., powder, tablet,
capsule, transdermal patch, implantable capsule, solution, emulsion). An appropriate
composition comprising a creatine analog and neuroprotective agent can be prepared
in a physiologically acceptable vehicle or carrier. For example, a composition in
tablet form can include one or more additives such as a filler (e.g., lactose), a binder
30 (e.g., gelatin, carboxymethylcellulose, gum arabic), a flavoring agent, a coloring
agent, or coating material as desired. For solutions or emulsions in general, carriers
may include aqueous or alcoholic/aqueous solutions, emulsions or suspensions,
including saline and buffered media. Parenteral vehicles can include sodium
chloride, solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's
35 or fixed oils. In addition, intravenous vehicles can include fluid and nutrient
replenishers, and electrolyte replenishers, such as those based on Ringer's dextrose.
Preservatives and other additives can also be present. For example, antimicrobial,
antioxidant, chelating agents, and inert gases can be added. (See, generally,
Remington's Pharmaceutical Sciences, 16th Edition, Mack, Ed., 1980).

5 The term "administration" is intended to include routes of administration
which allow the creatine compound/neuroprotective agent to perform their intended
function(s) of preventing, ameliorating, arresting, and/or eliminating disease(s) of the
nervous system in a subject. Examples of routes of administration which may be
10 etc.), oral, inhalation, transdermal, and rectal. Depending on the route of
administration, the creatine/neuroprotective agent may be coated with or in a material
to protect it from the natural conditions which may detrimentally effect its ability to
perform its intended function. The administration of the creatine/neuroprotective
agent is done at dosages and for periods of time effective to reduce, ameliorate or
15 eliminate the symptoms of the nervous system disorder. Dosage regimes may be
adjusted for purposes of improving the therapeutic or prophylactic response of the
compound. For example, several divided doses may be administered daily or the
dose may be proportionally reduced as indicated by the exigencies of the therapeutic
situation.

20

In addition, the methods of the instant invention comprise creatine
compounds effective in crossing the blood-brain barrier.

25 The creatine compounds/neuroprotective agents of this invention may be
administered alone or as a mixture with other creatine compounds, or together with
an adjuvant or other drug. For example, the creatine compound/neuroprotective
agent may be coadministered with other different art-recognized moieties such as
nucleotides, neurotransmitters, agonists or antagonists, steroids, immunomodulators,
immunosuppressants, vitamins, endorphins or other drugs which act upon the
30 nervous system or brain.

Creatine Kinase Isoenzymes in the Brain

35 Cells require energy to survive and to carry out the multitude of tasks that
characterize biological activity. Cellular energy demand and supply are generally
balanced and tightly regulated for economy and efficiency of energy use. Creatine
kinase plays a key role in the energy metabolism of cells with intermittently high and
fluctuating energy requirements such as skeletal and cardiac muscle, brain and neural
tissues, including, for example, the retina, spermatozoa and electrocytes. As stated
40 above, the enzyme catalyzes the reversible transfer of the phosphoryl group from
creatine phosphate to ADP, to generate ATP. There are multi-isoforms of creatine

- 5 kinase (CK) which include muscle (CK-MM), brain (CK-BB) and mitochondrial (CK-Mia, CK-Mib) isoforms.

Experimental data suggest that CK is located near the sites in cells where energy generation occurs; e.g., where force generation by motor proteins takes place,
10 next to ion pumps and transporters in membranes and where other ATP-dependent processes take place. It seems to play a complex multi-faceted role in cellular energy homeostasis. The creatine kinase system is involved in energy buffering/energy transport activities. It also is involved in regulating ADP and ATP levels intracellularly as well as ADP/ATP ratios. Proton buffering and production of
15 inorganic phosphate are important parts of the system.

In the brain, this creatine kinase system is quite active. Regional variations in CK activity with comparably high levels in cerebellum were reported in studies using native isoenzyme electrophoresis, or enzymatic CK activity measurements in either
20 tissue extracts or cultured brain cells. Chandler et al. *Stroke*, 19: 251-255 (1988), Maker et al. *Exp. Neurol.*, 38: 295-300 (1973), Manos et al. *J. Neurol. Chem.*, 56: 2101-2107 (1991). In particular, the molecular layer of the cerebellar cortex contains high levels of CK activity (Kahn *Histochem.*, 48: 29-32 (1976) consistent with the recent 31P-NMR findings which indicate that gray matter shows a higher flux through
25 the CK reaction and higher creatine phosphate concentrations as compared to white matter (Cadoux-Hudson et al. *FASEBJ.*, 3:2660-2666 (1989), but also high levels of CK activity were shown in cultured oligodendrocytes (Molloy et al. *J. Neurochem.*, 59:1925-1932 (1992), typical glial cells of the white matter. The brain CK isoenzyme CK-BB is the major isoform found in the brain. Lower amounts of
30 muscle creatine kinase (CK-MM) and mitochondrial creatine kinase (CK-Mi) are found.

Localization and Function of CK Isoenzymes in Different Cells of the Nervous System

35

Brain CK (CK-BB) is found in all layers of the cerebellar cortex as well as in deeper nuclei of the cerebellum. It is most abundant in Bergmann glial cells (BGC) and astroglial cells, but is also found in basket cells and neurons in the deeper nuclei. Hemmer et al., *Eur. J. Neuroscience*, 6:538-549 (1994), Hemmer et al. *Dev.*
40 *Neuroscience*, 15:3-5 (1993). The BGC is a specialized type of astroglial cell. It provides the migratory pathway for granule cell migration from the external to the

5 internal granule cell layer during cerebellar development. Another main function of these cells is the proposed ATP dependent spatial buffering of potassium ions released during the electrical activity of neurons (Newman et al. *Trends Neuroscience*, 8:156-159 (1985), Reichenbach, *Acad. Sci New York*, (1991), 272-286. Hence, CK-BB seems to be providing energy (ATP) for migration as well
10 as K^+ buffering through regulation of the Na^+/K^+ ATPase. The presence of CK-BB in astrocytes may be related to the energy requirements of these cells for metabolic interactions with neurons; e.g., tricarboxylic acid cycle (TCA) metabolite and neurotransmitter trafficking. Hertz, *Can J. Physiol. Pharmacol.*, 70: 5145-5157 (1991).

The Purkinje neurons of the cerebellum play a very important role in brain function. They receive excitatory input from parallel fibers and climbing fibers, they represent the sole neuronal output structures of the cerebellar cortex. Calcium mediated depolarizations in Purkinje cell dendrites are thought to play a central role in the mechanism of cerebellar motoric learning. Ito *Corr. Opin. Neurobiol.*, 1:616-620 (1991). High levels of muscle CK (CK-MM) were found in Purkinje neurons. There is strong evidence to support that CK-MM is directly or indirectly coupled to energetic processes needed for Ca^{++} homeostasis or to cellular processes triggered by this second messenger.

25 The glomerular structures of the cerebellum contain high levels of CK-BB and mitochondrial CK (CK-Mi). Large amounts of energy are needed in these structures for restoration of potassium ion gradients partially broken down during neuronal excitation as well as for metabolic and neurotransmitter trafficking between
30 glial cells and neurons. The presence of CK in these structures may be an indication that part of the energy consumed in these giant complexes might be supported by the creatine kinase system.

In neurons, CK-BB is found in association with synaptic vesicles (Friedhoff
35 and Lerner, *Life Sci.*, 20:867-872 (1977) as well as with plasma membranes (Lim et
al., *J. Neurochem.*, 41: 1177-1182 (1983)).

There is evidence to suggest that CK is bound to synaptic vesicles and to the plasma membrane in neurons may be involved in neurotransmitter release as well as in the maintenance of membrane potentials and the restoration of ion gradients before and after stimulation. This is consistent with the fact that high energy turnover and

5 concomitantly high CK concentrations have been found in those regions of the brain
that are rich in synaptic connections; e.g., in the molecular layer of the cerebellum, in
the glomerular structures of the granule layer and also in the hippocampus. The
observation that a rise in CK levels observed in a fraction of brain containing nerve
10 endings and synapses, parallels the neonatal increase in Na^+/K^+ ATPase is also
suggestive that higher levels of creatine phosphates and CK are characteristic of
regions in which energy expenditure for processes such as ion pumping are large.
Erecinska and Silver, *J. Cerebr. Blood Flow and Metabolism*, 9:2-19 (1989). In
addition, protein phosphorylation which plays an important role in brain function is
also through to consume a sizable fraction of the total energy available in those cells
15 (Erecinska and Silver, *id.* 1989). Finally, CK, together with nerve-specific enolase
belongs to a group of proteins known as slow component b (SCb). These proteins
are synthesized in neuronal cell body and are directed by axonal transport to the
axonal extremities. Brady and Lasek, *Cell*, 23: 515-523 (1981), Oblinger et al., *J.*
Neurol., 7: 433-462 (1987) The question of whether CK participates in the actual
20 energetics of axonal transport remains to be answered.

In conclusion, the CK system plays a key role in the energetics of the adult
brain. This is supported by ^{31}P NMR magnetization transfer measurements showing
that the pseudo first order rate constant of the CK reaction in the direction of ATP
25 synthesis as well as CK flux correlate with brain activity which is measured by EEG
as well as by the amount of deoxyglucose phosphate formed in the brain after
administration of deoxyglucose. The present inventors have discovered that diseases
of the nervous system can be treated by modulating the activity of the creatine
kinase/creatine phosphate pathway.

30

The Role of Creatine Kinase in Treating Diseases of the Nervous System

The mechanisms by which nerve cell metabolites are normally directed to
specific cell tasks is poorly understood. It is thought that nerve cells, like other cells,
regulate the rate of energy production in response to demand. The creatine kinase
35 system is active in many cells of the nervous system and is thought to play a role in
the allocation of high energy phosphate to many diverse neurological processes, such
as neurotransmitter biosynthesis, electrolyte flux and synaptic communication.
Neurological function requires significant energy and creatine kinase appears to play
an important role in controlling the flow of energy inside specialized excitable cells
40 such as neurons. The induction of creatine kinase, the BB isozyme and the brain
mitochondrial creatine kinase in particular, results in the generation of a high energy

5 state which could sustain or multiply the pathological process in diseases of the
nervous system. Creatine kinase induction also causes release of abnormally
elevated cellular energy reserves which appear to be associated with certain diseases
of the nervous system. Conversely, suppression of the creatine kinase system, or
aberrances in it, induce a low energy state which could result in or assist in the death
10 in the process of all the nervous system.

The components of the creatine kinase/phosphocreatine system include the
enzyme creatine kinase, the substrates creatine and creatine phosphate, and the
transporter of creatine. Some of the functions associated with this system include
15 efficient regeneration of energy in cells with fluctuating and high energy demand,
phosphoryl transfer activity, ion transport regulation, cytoskeletal association,
nucleotide pool preservation, proton buffering, and involvement in signal
transduction pathways. The creatine kinase/phosphocreatine system has been shown
to be active in neurons, astrocytes, oligodendrocytes, and Schwann cells. The
20 activity of the enzyme has been shown to be up-regulated during regeneration and
down-regulated in degenerative states, and aberrant in mitochondrial diseases.

Many diseases of the nervous system are thought to be associated with
abnormalities in an energy state which could result in imbalanced ion transport
25 neurotransmitter release and result in cell death. It has been reported that defects in
mitochondrial respiration enzymes and glycolytic enzymes may cause impairment of
cell function.

Without wishing to be bound by theory, it is thought that if the induction or
30 inhibition of creatine kinase is a cause or a consequence of disease, modulating its
activity, may block the disease. Modulating its activity would modulate energy flow
and affect cell function. Alternatively, another possibility is that creatine kinase
activity generates a product which affects neurological function. For example,
creatine phosphate may donate a phosphate to a protein to modify its function (e.g.,
35 activity, location). If phosphocreatine is such a phosphate donor, creatine analogs
which are phosphorylatable or phosphocreatine analogs may competitively inhibit
the interaction of phosphocreatine with a target protein thereby directly or indirectly
interfering with nervous system functions. Alternatively, phosphorylatable creatine
analog with altered phosphoryl group transfer potential may tie up phosphate stores
40 preventing efficient transfer of phosphate to targets. A neurological disease could be
associated with down regulation of creatine kinase activity. In such cases,

5 replenishment of the substrates, e.g., creatine, creatine phosphate or a substrate
analog, which could sustain ATP production for an extended of time, with other
activators of the enzyme could be beneficial for treatment of the disease.

10 Ingestion of creatine analogs has been shown to result in replacement of
tissue phosphocreatine pools by synthetic phosphagens with different kinetic and
thermodynamic properties. This results in subtle changes of intracellular energy
metabolism, including the increase of total reserves of high energy phosphate (see
refs. Roberts, J.J. and J.B. Walker, *Arch Biochem. Biophys* 220(2): 563-571 (1983)).
The replacement of phosphocreatine pools with slower acting synthetic phosphagens,
15 such as creatine analogs might benefit neurological disorders by providing a longer
lasting source of energy. One such analog, cyclocreatine (1-carboxymethyl-2-
aminoimidazolidine) modifies the flow of energy of cells in stress and may interfere
with ATP utilization at sites of cellular work.

20 The pathogenesis of nerve cell death in neurodegenerative diseases is
unknown. A significant amount of data has supported the hypothesis that an
impairment of energy metabolism may underlie the slow exitotoxic neuronal death.
Several studies have demonstrated mitochondrial or oxidative defects in
neurodegenerative diseases. Impaired energy metabolism results in decreases in high
25 energy phosphate stores and a deteriorating membrane potential. Under these
conditions the voltage sensitive Mg^{2+} block of NMDA receptors is relieved, allowing
the receptors to be persistently activated by endogenous concentrations of glutamate.
In this way, energy related metabolic defects may lead to neuronal death by a slow
exitotoxic mechanism. Recent studies indicate that such a mechanism occurs *in vivo*,
30 and it may play a role in animal models of Huntington's disease and Parkinson's
disease.

As discussed in detail above, the creatine kinase/ creatine phosphate energy
system is only one component of an elaborate energy-generating system found in the
35 nervous system. The reaction catalyzed by this system results in the rapid
regeneration of energy in the form of ATP at sites of cellular work. In the
mitochondria the enzyme is linked to the oxidative phosphorylation pathway that has
been implicated in diseases of the nervous system. There the enzyme works in the
reverse direction where it stores energy in the form of creatine phosphate.

40

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5 The invention is further illustrated in the following examples which in no way
should be construed as being further limiting. These examples provide evidence that
creatine compounds, represented by creatine itself and the analogue cyclocreatine,
are neuroprotective agents in animal models used for neurodegenerative diseases,
specifically, Huntington's disease and Parkinson's disease. The contents of all
10 references, pending patent applications and published patent applications, cited
throughout this application (including the background section) are hereby
incorporated by reference. For example, all teachings with regard to creatine
compounds, ATP enhancing agents, neuroprotective agents, etc. are intended to be
part of the present invention. It should be understood that the models used
15 throughout the examples are accepted models and that the demonstration of efficacy
in these models is predictive of efficacy in humans.

Examples

20 Example 1: Models for Huntington's Disease: Malonate and 3-Nitropropionic
Acid

 There is substantial evidence that energy production may play a role in the
pathogenesis of neurodegenerative diseases (Beal et al., *Ann. Neurol.* 31:119-130
(1992)). Impaired energy production may lead to activation of excitatory amino acid
25 receptors, increases in intracellular calcium and the generation of free radicals (Beal
et al., *Ann. Neurol.* 38:357-366 (1995)). In Huntington's Disease (HD) there is
reduced mitochondrial complex II-III activity in post mortem tissue and increased
cerebral lactate concentrations *in vivo* (Browne et al., *Ann. Neurol.*, in press, (1997);
Gu et al., *Ann. Neurol.* 39:385-389 (1996); Jenkins et al., *Neurology* 43 :2689-2695
30 (1993)).

 Animal models of Huntington's disease involve defects in energy production.
Malonate and 3-nitropropionic acid (3-NP) are, respectively, reversible and
irreversible inhibitors of complex II (succinate dehydrogenase) which produce
35 striatal lesions similar to those of HD (Beal et al., *J. Neurochem.* 61:1147-1150
(1993); Brouillet et al., *PNAS* 92:7105-7109 (1995); Henshaw et al., *Brain Research*
647:161-166 (1994)). The pathogenesis of lesions produced by these compounds
involves energy depletion, followed by activation of excitatory amino acid receptors
and free radical production (Schulz et al., *J. Neurosci.* 15:8419-8429 (1995); Schulz
40 et al., *J. Neurochem.* 64:936-939 (1995)).

5 The enzyme succinate dehydrogenase plays a central role in both the tricarboxylic acid cycle and the electron transport chain in the mitochondria. Intrastriatal injections of malonate in rats were shown to produce dose dependent striatal excitotoxic lesions which are attenuated by both competitive and non-competitive NMDA antagonists (Henshaw et al., *Brain Res.* 647:161-166 (1994)).

10 Furthermore, the glutamate release inhibitor lamotrigine also attenuates the lesions. Co-injection with succinate blocks the lesions consistent with an effect on succinate dehydrogenase. The lesions are accompanied by a significant reduction of ATP levels as well as significant increase in lactate levels *in vivo* as shown by chemical shift resonance imaging (Beal et al., *J. Neurochem* 61:1147-1150 (1993)).

15 Furthermore, the increases in lactate are greater in older animals consistent with a marked age of the lesion. Histological studies have shown that the lesion spares NADPH-diaphorase neurons. Somatostatin concentrations were also spared. *In vivo* magnetic resonance imaging of lesions shows a significant correlation between increasing lesion size and lactate production.

20 A series of experiments demonstrated that the administration of Q 10 or nicotinimide produced dose dependent protection against the lesions in the malonate animal model. These compounds attenuated ATP depletion produced by malonate *in vivo*. Furthermore, the co-administration of Q 10 with nicotinimide attenuated the
25 lesions and reduced increases in lactate which occurred after intrastriatal malonate injections.

All of the above mentioned studies supported malonate and 3-NP as useful models for the neuropathologic and neurochemical features of HD. The lesions produced similar patterns of cellular sparing seen in HD. There is a depletion of striatal spiny neurons, yet a relative preservation of the NADPH diaphorase interneurons. Furthermore, there is an increase in lactate concentration which has been observed in HD.

Oral administration of creatine and its analogue cyclocreatine were examined to determine their ability to attenuate malonate lesions. Creatine was administered orally to rats in their feed at doses of 0.25-3.0% of the diet. Cyclocreatine was administered at 0.2-1.0%. Controls received unsupplemented otherwise identical diets. The compounds were administered for two weeks prior to the administration of malonate and then for a further week prior to sacrifice. Malonate was dissolved in distilled deionized water and the pH was adjusted to 7.4 with 0.1 M HCl.

5 Intrastriatal injections of 1.5 ul of malonate containing 3 μ mol were made into the
striatum at the level of the bregma 2.4 mm lateral to the midline and 4.5 mm ventral
to the dura. Animals were sacrificed at 7 days by decapitation, and the brains were
quickly removed and placed in ice cold 0.9% saline solution. Brains were sectioned
10 at 2 mm intervals. Slices were then placed posterior side down in 2% 2,3,5-
triphenyltetrazolium chloride. Slices were stained in the dark at room temperature
for 30 minutes and then removed and placed in 4% paraformaldehyde, pH 7.3.
Lesions, noted by pale staining, were evaluated on the posterior surface of each
section using a Bioquant 4 system by an experienced histologist blinded to
experimental conditions. These measurements have been validated by comparing
15 them to measurements obtained on adjacent Nissl stain sections.

It was found that oral supplementation with both creatine and cyclocreatine
protected against striatal malonate lesions. A dose response curve for
neuroprotection by both creatine and cyclocreatine against malonate induced striatal
20 lesions was then examined. As shown in Figure 2, increasing doses of creatine from
0.25-3% in the diet exerted dose dependent neuroprotective effects against malonate
induced striatal lesions. Significant protection occurred with doses of 1% and 2% in
the diet. There was less protection at 3% creatine, suggesting that a U shaped dose
response may occur with higher doses. Administration of cyclocreatine resulted in
25 dose dependent neuroprotective effects which were significant at a dose of 1%
cyclocreatine.

In the 3-NP model, creatine was administered orally at a dose of 1% in feed.
Controls received unsupplemented rat chow. 3-NP was diluted in water and adjusted
30 to pH 7.4 with NaOH and administered at a dose of 10 mg/Kg intraperitoneally every
12 hours. Animals became acutely ill after 9-11 days. Since there was variability in
the times at which animals became ill, they were clinically examined 3 hours after
the injections and 1 animal of each group was sacrificed when an animal was acutely
ill, regardless of whether it was on a control diet or a creatine supplemented diet
35 (Schulz et al., *J. Neurochem.* 64:936-939 (1995)). Nine to ten animals were
examined in each group. Animals were sacrificed after showing acute illness and
striatal lesion volume was assessed by TTC staining as above. Statistical comparison
was made by student's t test.

40 A remarkable level of neuroprotection was seen against subacute 3-NP
neurotoxicity in creatine treated animals, as shown in Figure 3. Dietary

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5 supplementation with 1% creatine resulted in significant 83% reduction in lesion volume produced by 3-NP. This suggests that dietary supplementation with creatine may exert its greatest efficacy against more slowly evolving metabolic insults than against acute insults.

10 Example 2: MPTP as a model for Parkinson's Disease

MPTP, or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine is a neurotoxin which produces a Parkinsonian syndrome in both man and experimental animals. The initial report was by a chemist who was synthesizing and self injecting an opiate analogue. He inadvertently synthesized MPTP and developed profound
15 Parkinsonism. Subsequent pathologic studies showed severe degeneration in the pars compacta of the substantia nigra. A large outbreak subsequently occurred in California. These patients developed typical symptoms of Parkinsonism. They also had positron emission tomography done which showed a marked loss of dopaminergic innervation of the striatum.

20 Studies of the mechanism of MPTP neurotoxicity show that it involves the generation of a major metabolite, MPP⁺. This metabolite is formed by the activity of monoamine oxidase on MPTP. Inhibitors of monoamine oxidase block the neurotoxicity of MPTP in both mice and primates. The specificity of the neurotoxic effects of MPP⁺ for dopaminergic neurons appears to be due to the uptake of MPP⁺
25 by the synaptic dopamine transporter. Blockers of this transporter prevent MPP⁺ neurotoxicity. MPP⁺ has been shown to be a relatively specific inhibitor of mitochondrial complex I activity. It binds to complex I at the rotenone binding site. *In vitro* studies show that it produces an impairment of oxidative phosphorylation. *In vivo* studies have shown that MPTP can deplete striatal ATP concentrations in mice. It has been demonstrated that MPP⁺ administered intrastrially in rats produces significant depletion of ATP as well as increases in lactate confined to the striatum at the site of the injections. The present inventors have recently demonstrated that coenzyme Q₁₀, which enhances ATP production, can significantly protect against
30 MPTP toxicity in mice.

The effect of two representative creatine compounds, creatine and cyclocreatine, were evaluated using this model. Creatine and cyclocreatine were administered in the initial pilot experiment as 1% formulation in the feed of animals,
40 and was administered for three weeks before MPTP treatment. MPTP was administered intraperitoneally at a dose of 15mg/kg every 2 hours for five injections.

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5 The animals then remained on either creatine or cyclocreatine supplemented diets for
1 week before sacrifice. The mice examined were male Swiss Webster mice
weighing 30-35 grams obtained from Taconic Farms. Control groups received either
normal saline or MPTP hydrochloride alone. MPTP was administered in 0.1 ml of
10 water. The MPTP was obtained from Research Biochemicals. Eight to twelve
animals were examined in each group. Following sacrifice the two striata were
rapidly dissected and placed in chilled 0.1 M perchloric acid. Tissue was
subsequently sonicated, and aliquots were taken for protein quantification using a
fluorometer assay. Dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), and
15 homovanillic acid (HVA) were quantified by HPLC with 16 electrode
electrochemical detection. Concentrations of dopamine and metabolites were
expressed as nmol/mg protein. The statistical significance of differences was
determined by one-way ANOVA followed by Fisher PLSD post-hoc test to compare
group means.

20 The initial results are shown in Figure 4. Oral administration of either
cyclocreatine or creatine significantly protected against DOPAC depletions induced
by MPTP. Cyclocreatine was effective against MPTP induced depletions of
homovanillic acid. Both administration of creatine and cyclocreatine produce
significant neuroprotection against MPTP induced dopamine depletions. The
25 neuroprotective effect produced by cyclocreatine was greater than that seen with
creatine alone.

A dose response study was conducted where the creatine dose was 0.25%-
3.0% of the diet and cyclocreatine 0.25-1.0% of the diet. The results, shown in
30 Figure 5, demonstrate that doses of 0.25%, 0.5% and 1.0% creatine exerted dose-
dependent significant neuroprotection effects which disappeared at doses of 2.0%
and 3.0% creatine, consistent with a U shaped dose response curve. Cyclocreatine
exerted significant protection against dopamine depletions at 0.5% and 1.0%
cyclocreatine. Effects of creatine on the dopamine metabolites homovanillic acid
35 (HVA) and 3-4-dihydroxyphenyl acetic acid (DOPAC) paralleled those seen with
dopamine. Cyclocreatine also exerted neuroprotection effects against HVA and
DOPAC, although protection against HVA depletion was not seen with 0.5%
cyclocreatine which was suspected to be due to experimental variability.

40 These results indicate that the administration of creatine or cyclocreatine can
produce significant neuroprotective effects against MPTP induced dopaminergic

5 toxicity. These results imply that these compounds are useful for the treatment of
Parkinson's disease. The data further establish the importance of the creatine kinase
system in buffering energy and survival of neuronal tissue. Therefore, creatine
compounds which can sustain energy production in neurons are going to emerge as a
new class of protective agents of benefit therapeutically in the treatment of
10 neurodegenerative diseases where impairment of energy has been established.

Example 3: Effect of Dietary Creatine in a Mouse Model for ALS

Motor neuron degeneration was generated in mice that express a human Cu, Zn superoxide dismutase mutation. Gurney et al., *Science*, vol. 264, pp 1772-1775 (1994) These FALS mice develop a syndrome which mimics the symptoms of familial amyotrophic lateral sclerosis (FALS). Gradual loss of motor function becomes apparent, and typically the mice do not survive beyond 140 days.

FALS mice were divided into control and test groups. At approximately 80 days (between 70 and 90 days) after birth, the test groups (containing 5 mice per group) were changed over from a standard diet to a diet containing 1% creatine. The control group (containing 6 mice per group) were fed the standard diet.

Behavioral Testing-Rotorod

25 Mice were given two days to become acquainted with the rotarod apparatus
before testing began. Testing began with the animals trying to stay on a rod that was
rotating at 1 rpm. The speed was then increased by 1 rpm every 10 seconds until the
animal fell off. The speed of rod rotation at which the mouse fell off was used as the
measure of competency on this task. Animals were tested every other day until they
30 could no longer perform the task

The results for the test and control animals are shown in Figure 3. As shown in the Figure, the creatine-fed animals showed significantly better performance throughout the experiment suggesting less degeneration of motoneural skills than the control mice which were fed a standard diet.

Survival

FALS mice begin to show behavioral symptoms at about 120 days. The initial symptom is high frequency resting tremor. This progresses to gait abnormalities and uncoordinated movements. Later, the mice begin to show hemiparalysis of the hindlimbs, eventually progressing to paralysis of the forelimbs

5 and finally, complete paralysis. Animals in this study were sacrificed when they could no longer roll over within 10 seconds of being pushed on their side. This time point was taken as the time of death.

The results are shown graphically in Figure 4. Figure 4 shows that the animals placed on a diet containing 1% creatine survived longer than those placed on the control diet. Over 14 days of extension in survival was noted, which is a statistically significant improvement over the control mice.

The experiments performed on the FALS mice demonstrate that creatine has
15 beneficial effects as an additional therapy for ALS. It improves the quality of life
and extends survival.

Example 4: Neuroprotective Effects of Creatine and Nicotinamide against NMDA Mediated Excitotoxic Lesions

Materials and Methods

Studies of the neuroprotective effects of creatine and nicotinamide were carried out in 250 to 300 g male Sprague-Dawley rats. Creatine was administered orally to rats in their feed at a dose of 1% in the diet. Nicotinamide was administered orally with apple juice at a dose of 0.5% in the drinking water. Rats were treated for one week prior to intracerebral injections. Animals then remained on the control or supplemented diets for one week prior to being sacrificed. Eleven to 12 animals were examined in each experimental group. NMDA was administered at a dose of 240 nmol in 1 μ l. AMPA was administered at a dose of 30 nmol in 1 μ l and kainic acid was administered at dose of 5 nmol in 1 μ l. Malonate was dissolved in distilled deionized water and the pH was adjusted to 7.4 with HCl. Intrastriatal injections of 3 μ mol of malonate in 1.5 μ l were made with a 10 μ l Hamilton syringe fitted with a 26 gauge blunt tip needle, into the left striatum at the level of the bregma, 2.4 mm lateral to the midline and 4.5 mm ventral to the dura as described previously [Matthews, R.T *et al.* *J. Neurosci.*, 18 (1998) 156-163]. Following sacrifice the brains were quickly removed and placed in ice cold 0.9% saline solution. Brains were sectioned at 2 mm intervals throughout the rostro-caudal axis of the striatum. Slices were then placed posterior side down in 2% 2,3,5-triphenyltetrazolium chloride (TTC). Slices were stained in the dark at room temperature for 30 min and then removed and placed in 4% paraformaldehyde, pH 7.3. Lesions noted by pale staining were evaluated on the posterior surface of each section using a Bioquant 4 system, which calculates the volume of the lesions in each section.

5 by an experienced histologist blinded to experimental conditions. These measurements have been validated by comparing them with measurements obtained on adjacent Nissl stained sections. Statistical comparisons were made by unpaired t tests or by one-way analysis of variance followed by Fisher's protected least significant difference for post-hoc comparisons.

10

RESULTS

15 Creatine administration produced significant neuroprotective effects against striatal lesions produced by NMDA. There was no significant protection against either kainic acid or AMPA induced striatal excitotoxic lesions. Administration of nicotinamide alone produced a reduction in striatal lesion volume, however the reduction did not reach significance. Administration of creatine alone produced a significant neuroprotective effect against malonate lesions. The administration of nicotinamide with creatine produced additive neuroprotective effects which were greater than those seen
20 with either creatine or nicotinamide alone.

Previous studies have demonstrated that NMDA excitotoxic lesions are associated with impairment of both ATP and phosphocreatine levels [Bordelon *et al.* *J Neurochem*, 69 (1997) 1629-1639, Mitani, A., *et al.* *J Neurochem*, 62 (1994) 626-634].
25 There is also data that kainic acid lesions are associated with energy impairment. Lesions produced by NMDA however appear to be linked to mechanisms which differ from those which are associated with AMPA and kainic acid toxicity. An increase in calcium via activation of NMDA receptor is much more toxic than comparable increases caused by activation of voltage active calcium channels or kainic acid receptors (Tymianski *et al.* *J Neurosci*, 13 (1993) 2085-2104). Furthermore increased intracellular calcium following activation of NMDA receptors is associated with a much greater increase in free radical production than comparable increases produced by activation of kainate receptors or voltage dependent calcium channels (Dugan *et al.* *J. Neurosci.*, 15 (1995) 6377-6388, Reynolds *et al.* *J Neurosci*, 15 (1995) 3318-3327). Activation of NMDA
30 receptors is tied to a more rapid uptake of calcium into the mitochondria as compared to activation by voltage dependent calcium channels or by activation of AMPA or kainic acid receptors (Peng *et al.* *Mol Pharmacol*, 53 (1998) 974-980). Nitric oxide synthase inhibitors are effective in blocking NMDA excitotoxicity both in vitro and in vivo, whereas they are ineffective against both kainic acid and AMPA toxicity (Dawson *et al.* *Neurosci*, 13 (1993) 2651-2661). Specific coupling of NMDA receptors to nitric oxide neurotoxicity occurs by the NMDA receptor scaffolding protein PSD-95 (post-synaptic density-95) (Sattler *et al.* *Science*, 284 (1999) 1845-1848). Suppressing the expression of
40

5 PSD-95 attenuates excitotoxicity triggered by NMDA receptors, but not that produced by other glutamate receptors or calcium ion channels.

Creatine kinase along with its substrates creatine and phosphocreatine constitute an intricate cellular energy buffering and transport system connecting sites of energy production with sites of energy consumption (Hemmor *Dev. Neurosci.*, 15 (1993) 249-260). Creatine administration also stabilizes the mitochondrial creatine kinase and inhibits opening of the mitochondrial transition pore (O'Gorman *et al. FEBS Lett.*, 414 (1997) 253-2571). Creatine administration can also stimulate mitochondrial respiration and phosphocreatine synthesis (O'Gorman *et al. Biochim Biophys Acta*, 1276 (1996) 161-170). Phosphocreatine diffuses to the cytoplasm where it serves as both a temporal and spatial energy buffer maintaining ATP levels utilized by the sodium potassium ATPase and the calcium ATPase. Its importance to brain function is supported by *in vivo*³¹P NMR transfer measurements showing correlations of creatine kinase flux with brain activity as measured both by the EEG as well as brain 2deoxyglucose uptake (Corbett *et al J. Cereb. Blood Flow Metab.*, 14 (1994)1070-1077, Sauter *et al. J. Biol. Chem.*, 268 (1993) 13166-13171). Another potential mechanism by which phosphocreatine could inhibit excitotoxicity is by increasing glutamate uptake. Phosphocreatine serves as a direct energy source for glutamate uptake into synaptic vesicles (Xu *et al.J. Biol. Chem.*, 271 (1996) 13435-1344028). Lastly creatine kinase appears to be coupled directly or indirectly to energetic processes required for calcium homeostasis (Steehgs *et al. Cell*, 89 (1997) 93- 103). Creatine pretreatment delayed increases in intracellular calcium produced by 3-nitropropionic acid in cortical and striatal astrocytes *in vivo* (Deshpande *et al.Exp. Neurol.*, 145 (1997) 38-45). Administration of creatine may therefore improve intracellular calcium buffering and prevent free radical production by mitochondria. Creatine also protects mitochondrial creatine kinase from inactivation by peroxynitrite which is implicated in excitotoxic cell death (Stachowiak *et al. J Biol Chem*, 273 (1998)16694-16699). The present results suggest that stabilization of mitochondria and increasing mitochondrial PCr synthesis may be particularly effective against NMDA excitotoxicity as compared with that produced by nonNMDA receptor activation.

In the present study, it was also examined whether creatine could exert additive neuroprotective effects in combination with nicotinamide. It was found that creatine produced significant neuroprotective effects against malonate. A small protective effect of nicotinamide alone was found, although it did not reach statistical significance. The combination of nicotinamide with creatine however was more efficacious than the administration of either nicotinamide or creatine alone. Not to be limited by theory,

5 nicotinamide may be exerting neuroprotective effects either by increasing brain levels of
NADH which is a cofactor of the electron transport chain, or by inhibiting the activation
of polyADP-ribose polymerase which can lead to a depletion of intracellular ATP levels.
Creatine is neuroprotective against 3-nitropropionic acid and MPTP toxicity, and that
creatinine significantly extends survival in a transgenic mouse model of ALS (Klivenyi *et al.*
10 *Nature Med.*, 5 (1999) 347-350, Matthews *et al. Exp Neurol*, 157 (1999) 142-149).
The present studies provide further evidence that creatine exerts neuroprotective effects
in vivo. Oral supplementation with creatine or creatine in combination with
nicotinamide may therefore represent a novel therapeutic strategy for a number of
neurodegenerative diseases.

15 Creatine administration may be able to increase intracellular energy stores and to
inhibit activation of mitochondrial permeability transition. It was found that
administration of creatine in the diet significantly protected against NMDA excitotoxic
lesions. In addition, creatine produced significant protection against malonate induced
striatal lesions and exerted additive effects against these lesions when combined with
20 nicotinamide.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

Claims

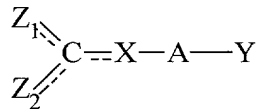
1. A method of increasing ATP production in the brain of a subject, comprising administering to a subject an effective amount of a creatine compound and an ATP enhancing agent, such that the ATP production in the brain is increased.

2. The method of claim 1, wherein said creatine compound is creatine.

3. The method of claim 1, wherein said creatine compound is cyclocreatine.

4. The method of claim 1, wherein said creatine compound is creatine phosphate.

5. The method of claim 1, wherein said creatine compound has the formula:



and pharmaceutically acceptable salts thereof, wherein:

a) Y is selected from the group consisting of: $-\text{CO}_2\text{H}$, $-\text{NHOH}$, $-\text{NO}_2$, $-\text{SO}_3\text{H}$, $-\text{C}(=\text{O})\text{NHSO}_2\text{J}$ and $-\text{P}(=\text{O})(\text{OH})(\text{OJ})$, wherein J is selected from the group consisting of: hydrogen, C_1 - C_6 straight chain alkyl, C_3 - C_6 branched alkyl, C_2 - C_6 alkenyl, C_3 - C_6 branched alkenyl, and aryl;

b) A is selected from the group consisting of: C, CH, C₁-C₅alkyl, C₂-C₅alkenyl, C₂-C₅alkynyl, and C₁-C₅ alkoyl chain, each having 0-2 substituents which are selected independently from the group consisting of:

1) K, where K is selected from the group consisting of: C₁-C₆ straight alkyl, C₂-C₆ straight alkenyl, C₁-C₆ straight alkoyl, C₃-C₆ branched alkyl, C₃-C₆ branched alkenyl, and C₄-C₆ branched alkoyl, K having 0-2 substituents independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

2) an aryl group selected from the group consisting of: a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents independently selected from the group consisting of: $-CH_2L$ and $-COCH_2L$ where L is

5 independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;
and

3) -NH-M, wherein M is selected from the group consisting of:
hydrogen, C₁-C₄ alkyl, C₂-C₄ alkenyl, C₁-C₄ alkoyl, C₃-C₄ branched alkyl, C₃-C₄
10 branched alkenyl, and C₄ branched alkoyl;

c) X is selected from the group consisting of NR₁, CHR₁, CR₁, O and S, wherein R₁ is selected from the group consisting of:

15 1) hydrogen;

2) K where K is selected from the group consisting of: C₁-C₆ straight alkyl, C₂-C₆ straight alkenyl, C₁-C₆ straight alkoyl, C₃-C₆ branched alkyl, C₃-C₆ branched alkenyl, and C₄-C₆ branched alkoyl, K having 0-2 substituents independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

3) an aryl group selected from the group consisting of a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents independently selected from the group consisting of: $-CH_2L$ and $-COCH_2L$ where L is independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

4) a C₅-C₉ α-amino-ω-methyl-ω-adenosylcarboxylic acid attached via the ω-methyl carbon;

30 5) a C₅-C₉ a-amino-w-aza-w-methyl-w-adenosylcarboxylic acid
attached via the w-methyl carbon; and

6) a C₅-C₉ α-amino-ω-thia-ω-methyl-ω-adenosylcarboxylic acid attached via the ω-methyl carbon;

35

d) Z_1 and Z_2 are chosen independently from the group consisting of: $=0$, $-NHR_2$, $-CH_2R_2$, $-NR_2OH$; wherein Z_1 and Z_2 may not both be $=0$ and wherein R_2 is selected from the group consisting of:

40 1) hydrogen;

- 5 2) K, where K is selected from the group consisting of: C₁-C₆ straight alkyl; C₂-C₆ straight alkenyl, C₁-C₆ straight alkoyl, C₃-C₆ branched alkyl, C₃-C₆ branched alkenyl, and C₄-C₆ branched alkoyl, K having 0-2 substituents independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;
- 10 3) an aryl group selected from the group consisting of a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents independently selected from the group consisting of: -CH₂L and -COCH₂L where L is independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;
- 15 4) a C₄-C₈ α-amino-carboxylic acid attached via the α-carbon;
- 5) B, wherein B is selected from the group consisting of: -CO₂H, -NHOH, -SO₃H, -NO₂, OP(=O)(OH)(OJ) and -P(=O)(OH)(OJ), wherein J is selected from the group consisting of: hydrogen, C₁-C₆ straight alkyl, C₃-C₆ branched alkyl, 20 C₂-C₆ alkenyl, C₃-C₆ branched alkenyl, and aryl, wherein B is optionally connected to the nitrogen via a linker selected from the group consisting of: C₁-C₂ alkyl, C₂ alkenyl, and C₁-C₂ alkoyl;
- 6) -D-E, wherein D is selected from the group consisting of: C₁-C₃ 25 straight alkyl, C₃ branched alkyl, C₂-C₃ straight alkenyl, C₃ branched alkenyl, C₁-C₃ straight alkoyl, aryl and aroyl; and E is selected from the group consisting of: -(P(=O))_nNMP, where n is 0-2 and NMP is ribonucleotide monophosphate connected via the 5'-phosphate, 3'-phosphate or the aromatic ring of the base; -[P(=O)(OCH₃)(O)]_m-Q, where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of 30 the base; -[P(=O)(OH)(CH₂)]_m-Q, where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base; and an aryl group containing 0-3 substituents chosen independently from the group consisting of: Cl, Br, epoxy, acetoxy, -OG, -C(=O)G, and -CO₂G, where G is independently selected from the group consisting of: C₁-C₆ straight alkyl, C₂-C₆ straight alkenyl, C₁-C₆ straight alkoyl, 35 C₃-C₆ branched alkyl, C₃-C₆ branched alkenyl, C₄-C₆ branched alkoyl, wherein E may be attached to any point to D, and if D is alkyl or alkenyl, D may be connected at either or both ends by an amide linkage; and
- 7) -E, wherein E is selected from the group consisting of - 40 (P(=O))_nNMP, where n is 0-2 and NMP is a ribonucleotide monophosphate connected via the 5'-phosphate, 3'-phosphate or the aromatic ring of the base; -[P(=O)(OCH₃)(O)]_m-Q,

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5 where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base; $-\text{[P(=O)(OH)(CH}_2\text{)]}_m\text{-Q}$, where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base; and an aryl group containing 0-3 substituents chose independently from the group consisting of: C₁, Br, epoxy, acetoxy, -OG, -C(=O)G, and -CO=G, where G is independently selected from the group
10 consisting of: C₁-C₆ straight alkyl, C₂-C₆ straight alkenyl, C₁-C₆ straight alkoyl, C₃-C₆ branched alkyl, C₃-C₆ branched alkenyl, C₄-C₆ branched alkoyl; and if E is aryl, E may be connected by an amide linkage;

e) if R₁ and at least one R₂ group are present, R₁ may be connected by a
15 single or double bond to an R₇ group to form a cycle of 5 to 7 members;

f) if two R₂ groups are present, they may be connected by a single or a double bond to form a cycle of 4 to 7 members; and

20 g) if R₁ is present and Z₁ or Z₂ is selected from the group consisting of -NHR₂, -CH₂R₂ and -NR₂OH, then R₁ may be connected by a single or double bond to the carbon or nitrogen of either Z₁ or Z₂ to form a cycle of 4 to 7 members.

6. The method of claim 1, wherein said ATP enhancing agent is CoQs, vitamins,
25 spin traps, carnitine, antioxidants, sugars, vincopocetine or combinations thereof.

7. The method of claim 6, wherein the agent is CoQ₁₀.

8. The method of claim 6, wherein the agent is carnitine.

9. The method of claim 6, wherein the sugar is ribose.

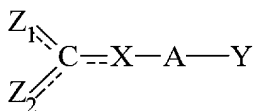
10. The method of claim 6, wherein said antioxidant is pyruvate.

35 11. The method of claim 6, wherein the antioxidant is lutein.

12. The method of claim 6, wherein the agent is vinpocetine.

13. The method of claim 1, further comprising administering a herbal extract.

- 5 14. The method of claim 13, wherein the extract is rosemary or black caraway extract.
15. The method of claim 1, further comprising administering a berry oil or meal.
- 10 16. The method of claim 15, wherein said berry oil or meal is from blackberries, blueberries, black raspberries, or mixtures thereof.
17. The method of claim 1, wherein said subject is suffering or at risk of suffering from a nervous system disorder.
- 15 18. The method of claim 1, wherein said subject is human.
19. A method of preventing nervous system disorders, comprising administering to a subject an effective amount of a creatine compounds and a neuroprotective agent, such that said nervous system disorders are prevented.
- 20 20. The method of claim 19, wherein said creatine compound is creatine.
21. The method of claim 19, wherein said creatine compound is cyclocreatine.
- 25 22. The method of claim 19, wherein said creatine compound is creatine phosphate.
23. The method of claim 19, wherein said creatine compound has the formula:



30

and pharmaceutically acceptable salts thereof, wherein:

- 35 a) Y is selected from the group consisting of: -CO₂H, -NHOH, -NO₂, -SO₃H, -C(=O)NHSO₂J and -P(=O)(OH)(OJ), wherein J is selected from the group consisting of: hydrogen, C₁-C₆ straight chain alkyl, C₃-C₆ branched alkyl, C₂-C₆ alkenyl, C₃-C₆ branched alkenyl, and aryl;

5 b) A is selected from the group consisting of: C, CH, C₁-C₅alkyl, C₂-C₅alkenyl, C₂-C₅alkynyl, and C₁-C₅ alkoyl chain, each having 0-2 substituents which are selected independently from the group consisting of:

1) K, where K is selected from the group consisting of: C₁-C₆ straight alkyl, C₂-C₆ straight alkenyl, C₁-C₆ straight alkoyl, C₃-C₆ branched alkyl, C₃-C₆ branched alkenyl, and C₄-C₆ branched alkoyl, K having 0-2 substituents independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

2) an aryl group selected from the group consisting of: a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents independently selected from the group consisting of: $-CH_2L$ and $-COCH_2L$ where L is independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy; and

20 3) -NH-M, wherein M is selected from the group consisting of:
hydrogen, C₁-C₄ alkyl, C₂-C₄ alkenyl, C₁-C₄ alkoyl, C₃-C₄ branched alkyl, C₃-C₄
branched alkenyl, and C₄ branched alkoyl;

c) X is selected from the group consisting of NR₁, CHR₁, CR₁, O and S,
25 wherein R₁ is selected from the group consisting of:

1) hydrogen;

2) K where K is selected from the group consisting of: C₁-C₆ straight alkyl, C₂-C₆ straight alkenyl, C₁-C₆ straight alkoyl, C₃-C₆ branched alkyl, C₃-C₆ branched alkenyl, and C₄-C₆ branched alkoyl, K having 0-2 substituents independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

35 3) an aryl group selected from the group consisting of a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents independently selected from the group consisting of: $-CH_2L$ and $-COCH_2L$ where L is independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

4) a C₅-C₉ α-amino-ω-methyl-ω-adenosylcarboxylic acid attached
40 via the ω-methyl carbon;

5 5) a C₅-C₉ a-amino-w-aza-w-methyl-w-adenosylcarboxylic acid
attached via the w-methyl carbon; and

6) a C₅-C₉ α-amino-ω-thia-ω-methyl-ω-adenosylcarboxylic acid attached via the ω-methyl carbon;

10

d) Z_1 and Z_2 are chosen independently from the group consisting of: $=0$, $-NHR_2$, $-CH_2R_2$, $-NR_2OH$; wherein Z_1 and Z_2 may not both be $=0$ and wherein R_2 is selected from the group consisting of:

15

1) hydrogen;

2) K, where K is selected from the group consisting of: C₁-C₆ straight alkyl; C₂-C₆ straight alkenyl, C₁-C₆ straight alkoyl, C₃-C₆ branched alkyl, C₃-C₆ branched alkenyl, and C₄-C₆ branched alkoyl, K having 0-2 substituents

20

independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

3) an aryl group selected from the group consisting of a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents independently selected from the group consisting of: $-CH_2L$ and $-COCH_2L$ where L is independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

25

4) a C₄-C₈ α-amino-carboxylic acid attached via the α-carbon;

5) B, wherein B is selected from the group consisting of: $-\text{CO}_2\text{H}$, $-\text{NHOH}$, $-\text{SO}_3\text{H}$, $-\text{N}_02$, $\text{OP}(=\text{O})(\text{OH})(\text{OJ})$ and $-\text{P}(=\text{O})(\text{OH})(\text{OJ})$, wherein J is selected from the group consisting of: hydrogen, C_1 - C_6 straight alkyl, C_3 - C_6 branched alkyl, C_2 - C_6 alkenyl, C_3 - C_6 branched alkenyl, and aryl, wherein B is optionally connected to the nitrogen via a linker selected from the group consisting of: C_1 - C_2 alkyl, C_2 alkenyl, and C_1 - C_2 alkoyl;

35

6) -D-E, wherein D is selected from the group consisting of: C₁-C₃ straight alkyl, C₃ branched alkyl, C₂-C₃ straight alkenyl, C₃ branched alkenyl, C₁-C₃ straight alkoyl, aryl and aroyl; and E is selected from the group consisting of:
 -(P(O₃)_n)NMP, where n is 0-2 and NMP is ribonucleotide monophosphate connected via the 5'-phosphate, 3'-phosphate or the aromatic ring of the base; -[P(=O)(OCH₃)(O)]_m-Q, where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of

the base; $-\text{P}(=\text{O})(\text{OH})(\text{CH}_2)_m-\text{Q}$, where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base; and an aryl group containing 0-3 substituents chosen independently from the group consisting of: Cl , Br , epoxy, acetoxy, $-\text{OG}$, $-\text{C}(=\text{O})\text{G}$, and $-\text{CO}_2\text{G}$, where G is independently selected from the group consisting of: $\text{C}_1\text{-C}_6$ straight alkyl, $\text{C}_2\text{-C}_6$ straight alkenyl, $\text{C}_1\text{-C}_6$ straight alkoyl, $\text{C}_3\text{-C}_6$ branched alkyl, $\text{C}_3\text{-C}_6$ branched alkenyl, $\text{C}_4\text{-C}_6$ branched alkoyl, wherein E may be attached to any point to D , and if D is alkyl or alkenyl, D may be connected at either or both ends by an amide linkage; and

7) -E, wherein E is selected from the group consisting of -
 (P(O)₃)_nNMP, where n is 0-2 and NMP is a ribonucleotide monophosphate connected via
 the 5'-phosphate, 3'-phosphate or the aromatic ring of the base; -[P(=O)(OCH₃)(O)]_m-Q,
 where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of
 the base; -[P(=O)(OH)(CH₂)]_m-Q, where m is 0-3 and Q is a ribonucleoside connected
 via the ribose or the aromatic ring of the base; and an aryl group containing 0-3
 substituents chosen independently from the group consisting of: C₁, Br, epoxy, acetoxy,
 -OG, -C(=O)G, and -CO=G, where G is independently selected from the group
 consisting of: C₁-C₆ straight alkyl, C₂-C₆ straight alkenyl, C₁-C₆ straight alkoyl, C₃-C₆
 branched alkyl, C₃-C₆ branched alkenyl, C₄-C₆ branched alkoyl; and if E is aryl, E may
 be connected by an amide linkage;

e) if R₁ and at least one R₂ group are present, R₁ may be connected by a single or double bond to an R₂ group to form a cycle of 5 to 7 members;

f) if two R₂ groups are present, they may be connected by a single or a double bond to form a cycle of 4 to 7 members; and

g) if R₁ is present and Z₁ or Z₂ is selected from the group consisting of -NHR₂, -CH₂R₂ and -NR₂OH, then R₁ may be connected by a single or double bond to the carbon or nitrogen of either Z₁ or Z₂ to form a cycle of 4 to 7 members.

24. The method of claim 19, wherein said nervous system disorder is selected from the group consisting of Alzheimer's, ALS, Huntington's, Multiple Sclerosis, and aging.

25. The method of claim 19, wherein said neuroprotective agent is selected from the group consisting of approved drugs for the prevention or treatment of neurodegenerative diseases, inhibitors of glutamate excitotoxicity, growth factors, nitric oxide synthase

5 inhibitors, cyclooxygenase 2 inhibitors, aspirin, ICE inhibitors, neuroimmunophilis, N-acetylcystine, antioxidants, vinpocetine, fatty acids, lipoic acid, vitamins, cofactors, and CoQ₁₀.

10 26. The method of claim 25, wherein the agent is CoQ₁₀.

27. The method of claim 25, wherein the fatty acid is docosahexanoic acid.

28. The method of claim 25, wherein the fatty acid is eicosapentenoic acid.

15 29. The method of claim 25, wherein the fatty acid is gamma linolenic acid.

30. The method of claim 25, further comprising administering a herbal extract.

20 31. The method of claim 30, wherein the extract is rosemary or black caraway extract.

32. The method of claim 19, further comprising administering a berry oil or meal.

25 33. The method of claim 32, wherein said berry oil or meal is from blackberries, blueberries, black raspberries, or mixtures thereof.

30 34. A method of protecting the nervous system of a subject against oxidative damage, comprising administering to said subject an effective amount of a creatine compound and a neuroprotective agent, such that the nervous system of the subject is protected against oxidative damage.

35 35. The method of claim 34, wherein said creatine compound is creatine.

36. The method of claim 34, wherein said creatine compound is cyclocreatine.

37. The method of claim 34, wherein said creatine compound is creatine phosphate.

40 38. The method of claim 34, wherein said neuroprotective agent is an anti-oxidant compound.

39. The method of claim 38, wherein said anti-oxidant is selected from the group consisting of vitamin E, lutein, pyruvate, alpha-omega fatty acids, BHP, alpha-lipoate, thioctic acid, 1,2-dithiolane-3-pentanoic acid, 1,2-dithiolane-3 valeric acid, and 6,8-dithiooctanoic acid.
40. A method of treating a subject suffering from a nervous system disorder, comprising administering to said subject a creatine kinase modulating compound which enhances ATP production and a neuroprotective agent, such that said nervous system disorder is treated.
41. The method of claim 40, wherein said creatine kinase modulating compound is a creatine compound.
42. The method of claim 40, wherein said creatine compound is creatine.
43. The method of claim 40 wherein said creatine compound is creatine phosphate.
44. The method of claim 40, wherein said creatine compound is cyclocreatine.
45. The method of claim 40, wherein said subject is suffering from a nervous system disorder selected from the group consisting of Alzheimer's, Multiple Sclerosis, ALS, or Huntington's disease.
46. The method of claim 45, wherein said neuroprotective agent is selected from the group consisting of approved drugs for the prevention or treatment of neurodegenerative diseases, inhibitors of glutamate excitotoxicity, growth factors, nitric oxide synthase inhibitors, cyclooxygenase 2 inhibitors, aspirin, ICE inhibitors, neuroimmunophilis, N-acetylcystine, antioxidants, vinpocetine, fatty acids, lipoic acid, vitamins, cofactors, and CoQ₁₀.
47. A method for protecting the nervous system against nervous system disease states comprising administering to a subject a dietary food supplement comprising a creatine compound and a neuroprotective agent.
48. The method of claim 47, wherein said method enhances nervous system activities.

5 49. The method of claim 48, wherein said nervous system activity is memory.

50. The method of claim 47, wherein said nervous system disease is Alzheimer's, Multiple Sclerosis, ALS, aging, or Huntington's disease.

10 51. The method of claim 47, wherein said neuroprotective agent is selected from the group consisting of approved drugs for the prevention or treatment of neurodegenerative diseases, inhibitors of glutamate excitotoxicity, growth factors, nitric oxide synthase inhibitors, cyclooxygenase 2 inhibitors, aspirin, ICE inhibitors, neuroimmunophilis, N-acetylcysteine, antioxidants, vinpocetine, fatty acids, lipoic acid, vitamins, cofactors, and
15 CoQ₁₀.

52. The method of claim 47, further comprising administering a herbal extract.

20 53. The method of claim 52, wherein the extract is rosemary or black caraway extract.

54. The method of claim 47, further comprising administering a berry oil or meal.

25 55. The method of claim 54, wherein said berry oil or meal is from blackberries, blueberries, black raspberries, or mixtures thereof.

30 56. A method for treating memory impairment in a subject, comprising administering to said subject an effective amount of a creatine kinase modulating compound and a neuroprotective agent, such that said memory impairment is treated in said subject

57. The method of claim 56, wherein said subject is administered a creatine kinase modulating compound to prevent memory impairment.

35 58. The method of claim 56, wherein said subject is suffering from Alzheimer's disease, ALS, or Huntington's disease.

59. The method of claim 56, wherein said creatine kinase modulating compound is a creatine compound.

40

60. The method of claim 59, wherein said creatine compound is creatine.

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61. The method of claim 59, wherein said creatine compound is creatine phosphate.
62. The method of claim 59, wherein said creatine compound is cyclocreatine.
63. The method of claim 56, wherein said neuroprotective agent is selected from the group consisting of approved drugs for the prevention or treatment of neurodegenerative diseases, inhibitors of glutamate excitotoxicity, growth factors, nitric oxide synthase inhibitors, cyclooxygenase 2 inhibitors, aspirin, ICE inhibitors, neuroimmunophilis, N-acetylcystine, antioxidants, vinpocetine, fatty acids, lipoic acid, vitamins, cofactors, and CoQ₁₀.

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Abstract of the Disclosure

The present invention relates to the use of creatine compound and neuroprotective combinations including creatine, creatine phosphate or analogs of creatine, such as cyclocreatine, for treating diseases of the nervous system. Creatine compounds in combination with neuroprotective agents can be used as therapeutically effective compositions against a variety of diseases of the nervous system such as diabetic and toxic neuropathies, peripheral nervous system diseases, Alzheimer disease, Parkinson's disease, stroke, Huntington's disease, amyotrophic lateral sclerosis, motor neuron disease, traumatic nerve injury, multiple sclerosis, dysmyelination and demyelination disorders, and mitochondrial diseases. The creatine compounds which can be used in the present method include (1) creatine, creatine phosphate and analogs of these compounds which can act as substrates or substrate analogs for creatine kinase; (2) bisubstrate inhibitors of creatine kinase comprising covalently linked structural analogs of adenosine triphosphate (ATP) and creatine; (3) creatine analogs which can act as reversible or irreversible inhibitors of creatine kinase; and (4) N-phosphorocreatine analogs bearing non-transferable moieties which mimic the N-phosphoryl group.

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LESION VOLUME (mm³)

30
25
20
15
10
5
0

CONTROL
1% CREATINE
1% CYCLOCREATINE

*

Group	Lesion Volume (mm ³)
CONTROL	~22.5
1% CREATINE	~12.5*
1% CYCLOCREATINE	~15.5

FIGURE 1

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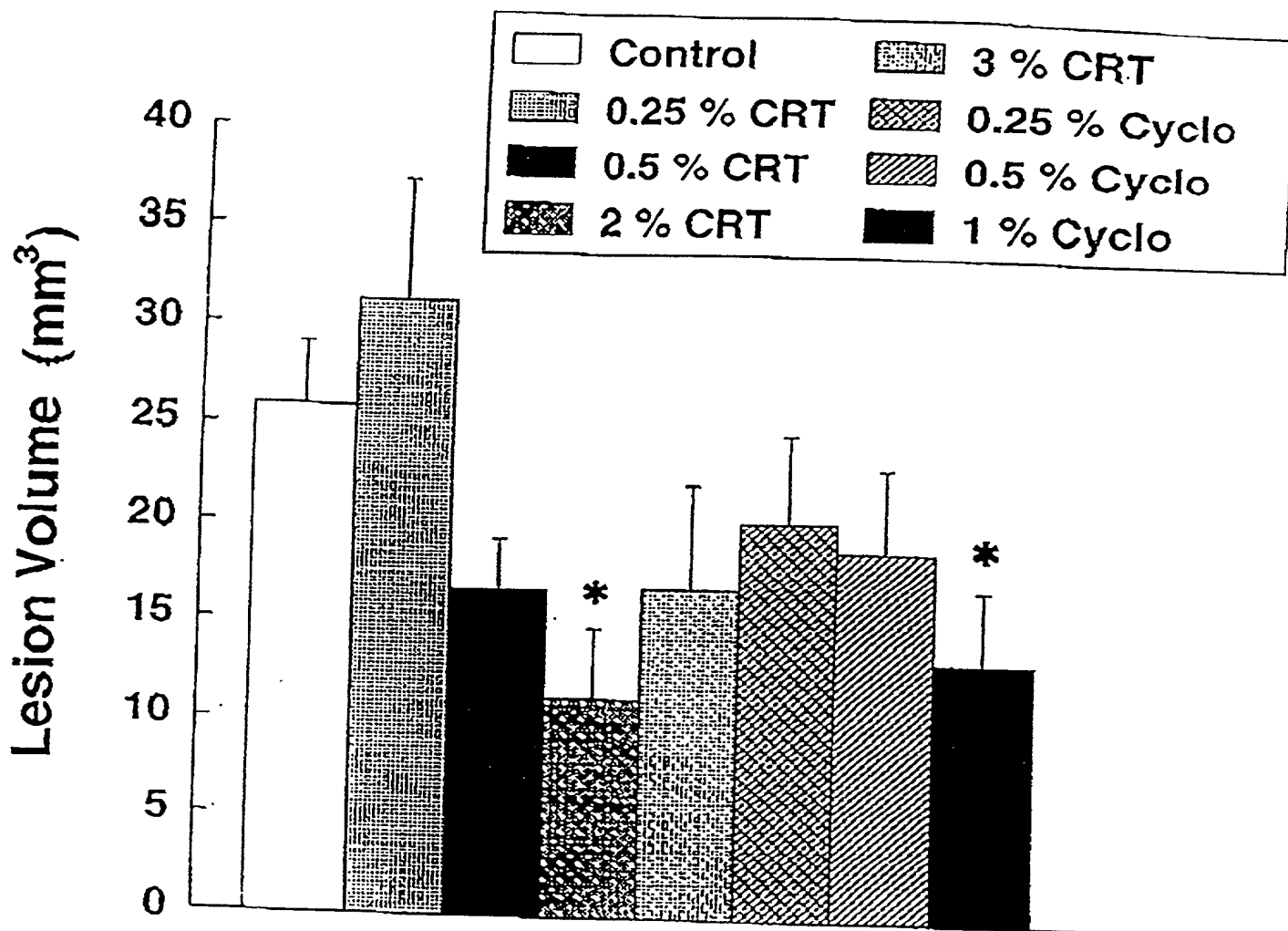


FIGURE 2

Bar graph showing Lesion Volume (mm³) for Control and 1% Creatine groups. The Control group has a lesion volume of approximately 67 mm³, while the 1% Creatine group has a significantly lower lesion volume of approximately 12 mm³. The difference is statistically significant (p < 0.05), indicated by three asterisks (*).

Group	Lesion Volume (mm ³)
Control	~67
1 % Creatine	~12

FIGURE 3

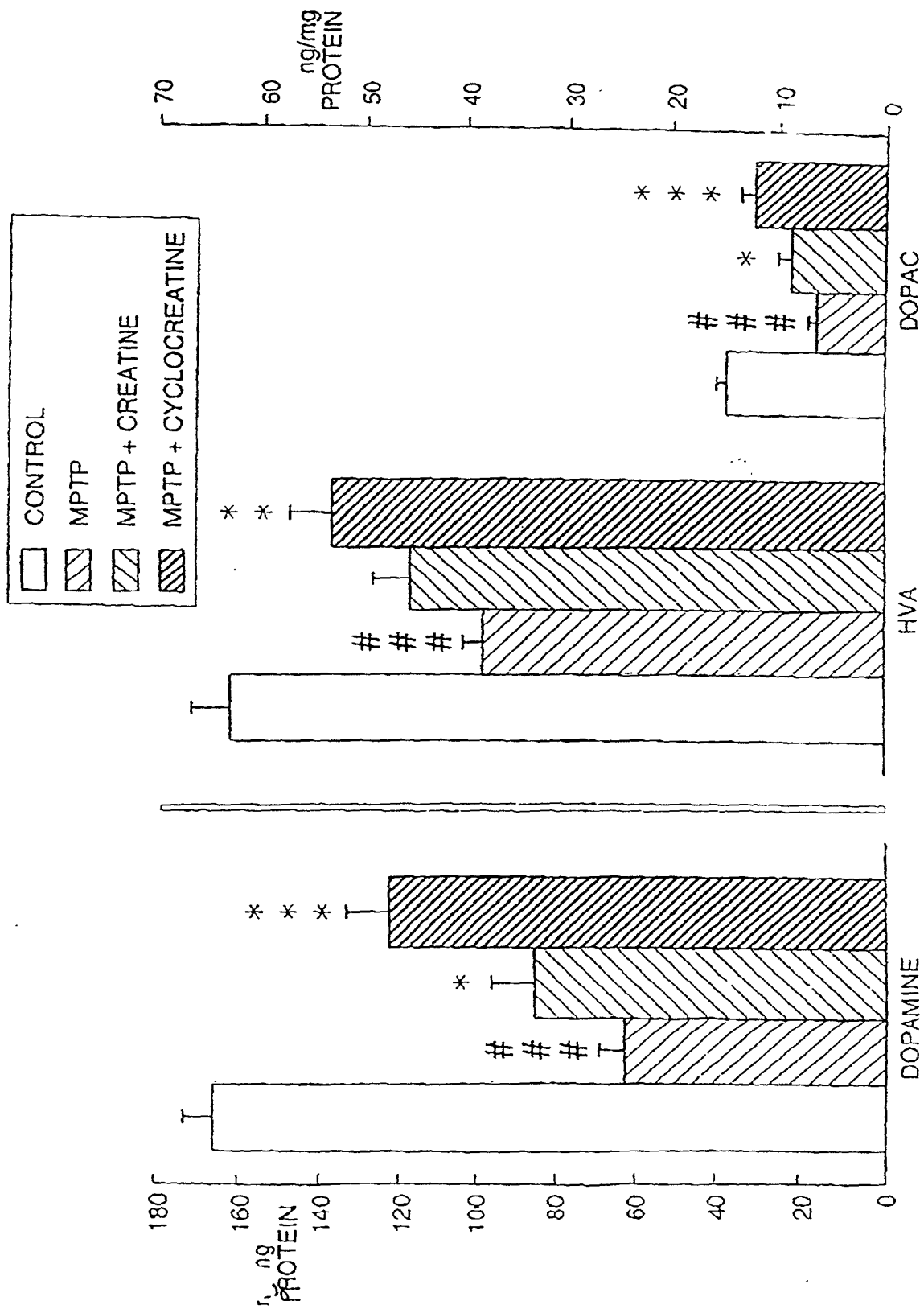


FIGURE 4

Dopamine

Treatment	Saline	MPTP	0.25% Creatine	0.5% Creatine	1% Creatine	2% Creatine	3% Creatine	0.25% Cyclo	0.5% Cyclo	1% Cyclo
ng/mg protein	~168	~168	~168	~168	~168	~168	~168	~168	~168	~168

HVA

Treatment	Saline	MPTP	0.25% Creatine	0.5% Creatine	1% Creatine	2% Creatine	3% Creatine	0.25% Cyclo	0.5% Cyclo	1% Cyclo
ng/mg protein	~168	~168	~168	~168	~168	~168	~168	~168	~168	~168

DOPAC

Treatment	Saline	MPTP	0.25% Creatine	0.5% Creatine	1% Creatine	2% Creatine	3% Creatine	0.25% Cyclo	0.5% Cyclo	1% Cyclo
ng/mg protein	~168	~168	~168	~168	~168	~168	~168	~168	~168	~168

DOPAC

Treatment	Saline	MPTP	0.25% Creatine	0.5% Creatine	1% Creatine	2% Creatine	3% Creatine	0.25% Cyclo	0.5% Cyclo	1% Cyclo
ng/mg protein	~168	~168	~168	~168	~168	~168	~168	~168	~168	~168

The figure consists of four bar graphs arranged in a 2x2 grid. The top row shows Dopamine and HVA levels, and the bottom row shows DOPAC levels. Each graph has a y-axis labeled 'ng/mg protein' ranging from 0 to 180. The x-axis lists ten treatment groups: Saline, MPTP, 0.25% Creatine, 0.5% Creatine, 1% Creatine, 2% Creatine, 3% Creatine, 0.25% Cyclo, 0.5% Cyclo, and 1% Cyclo. A legend box on the left side of the figure identifies each treatment group by its bar pattern. In all three graphs (Dopamine, HVA, and DOPAC), the MPTP treatment significantly reduces levels compared to Saline. The various creatine and cyclocreatine treatments show varying degrees of recovery or prevention of the MPTP-induced decrease. Statistical significance is indicated by asterisks (*) above certain bars.

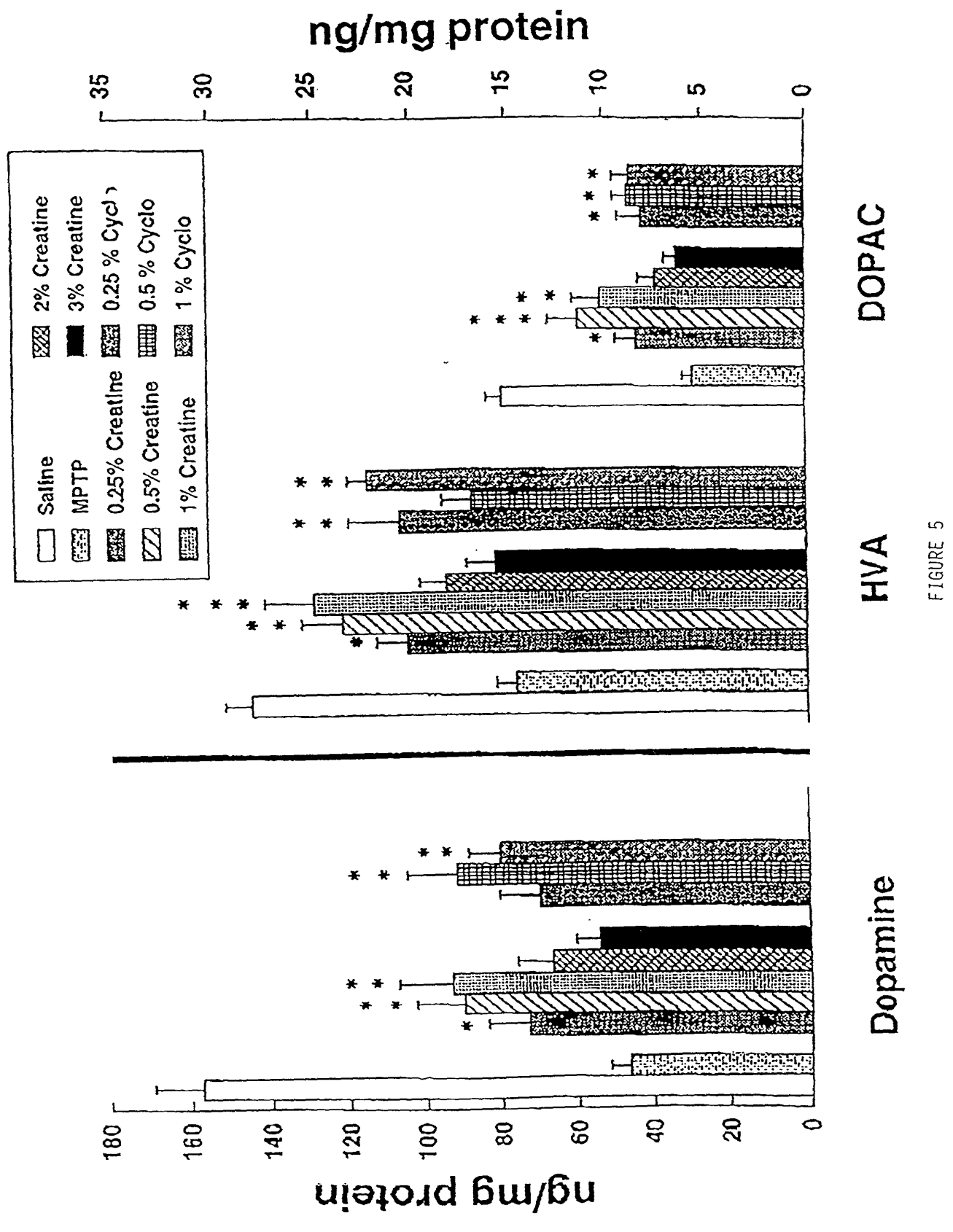
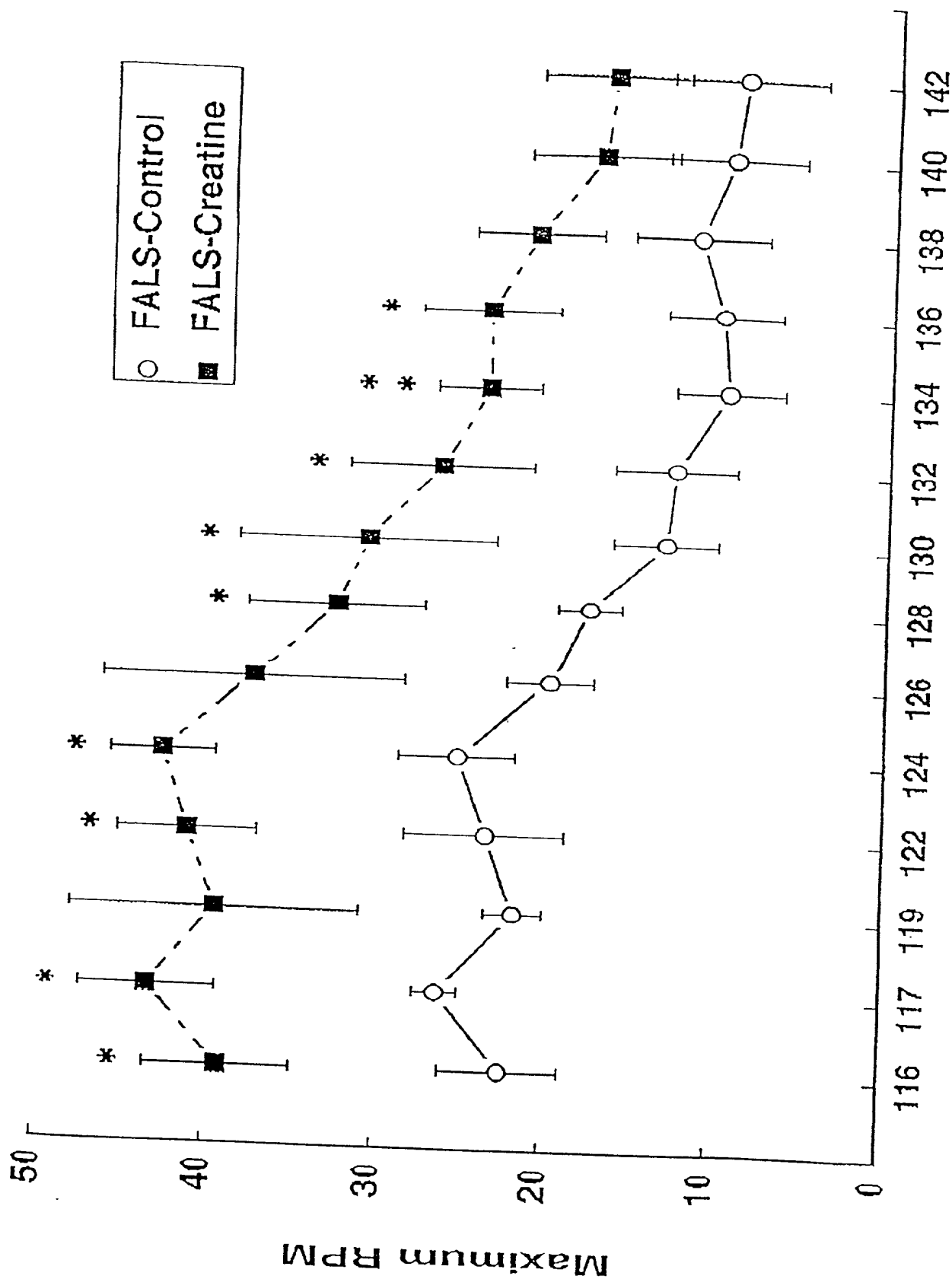


FIGURE 5



Days of Age

FIGURE 6

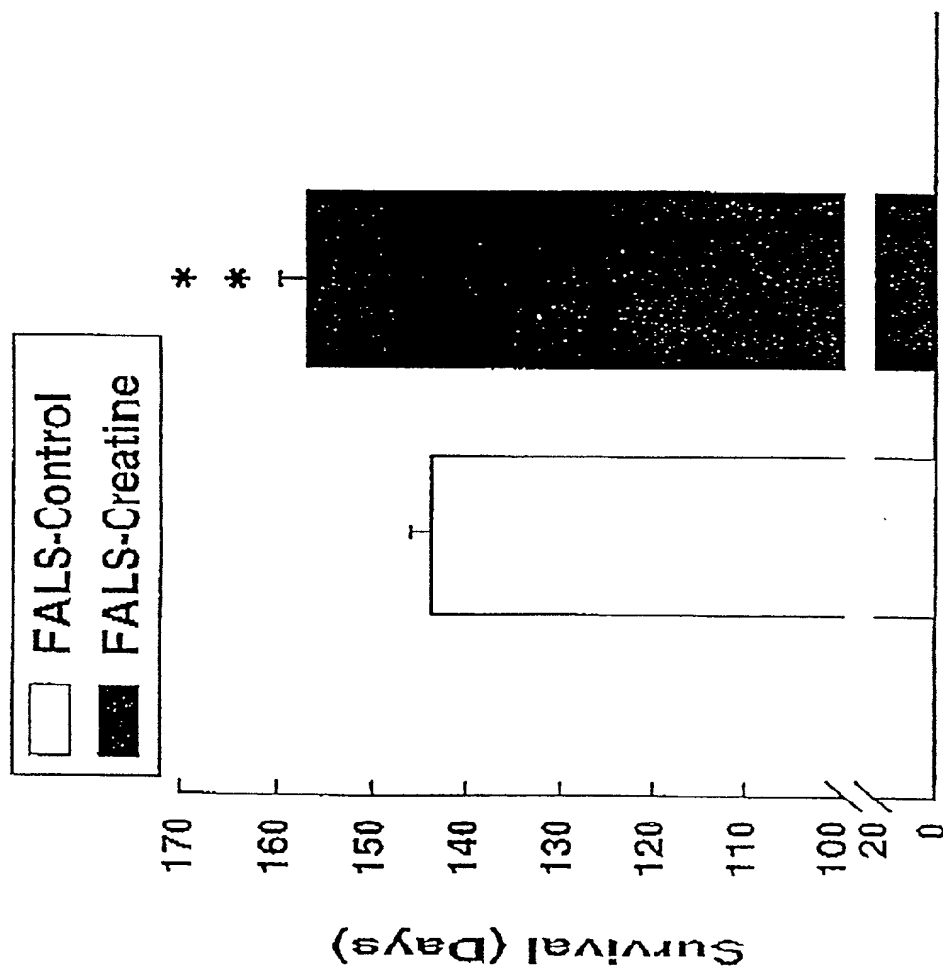


FIGURE 7

[illegible]

Attorney's
Docket
Number AVZ-007CP3

Declaration, Petition and Power of Attorney for Patent Application

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

COMPOSITIONS CONTAINING A COMBINATION OF A CREATINE COMPOUND
AND A SECOND AGENT
the specification of which

(check one)

X is attached hereto.

_____ was filed on _____ as _____

Application Serial No. _____

and was amended on _____
(if applicable)

I do not know and do not believe that the subject matter of this application was known or used by others in the United States or patented or described in a printed publication in any country before my invention thereof, or patented or described in a printed publication in any country or in public use or on sale in the United States more than one year prior to the date of this application, or first patented or caused to be patented or made the subject of an inventor's certificate by me or my legal representatives or assigns in a country foreign to the United States prior to the date of this application on an application filed more than twelve months (six months if this application is for a design) before the filing of this application; and I acknowledge my duty to disclose information of which I am aware which is material to the examination of this application, that no application for patent or inventor's certificate on the subject matter of this application has been filed by me or my representatives or assigns in any country foreign to the United States, except those identified below, and that I have reviewed and understand the contents of the specification, including the claims as amended by any amendment referred to herein.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

CLAIM OF BENEFIT OF EARLIER FOREIGN APPLICATION(S)

I hereby claim priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below, and have also identified below any foreign application(s) for patent or inventor's certificate filed by me on the same subject matter having a filing date before that of the application(s) from which priority is claimed.

Check one:

☒ no such applications have been filed.

such applications have been filed as follows

EARLIEST FOREIGN APPLICATION(S), IF ANY, FILED WITHIN 12 MONTHS
(6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

Country	Application Number	Date of Filing (month,day,year)	Priority Claimed Under 35 USC 119
			<input type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No

ALL FOREIGN APPLICATION(S), IF ANY FILED MORE THAN 12 MONTHS
(6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

CLAIM FOR BENEFIT OF U.S. PROVISIONAL APPLICATION(S)

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below.

<u>60/080,459</u>	<u>April 2, 1998</u>
(Application Serial No.)	(Filing Date)

<u>09/285,395</u>	<u>April 2, 1999</u>
(Application Serial No.)	(Filing Date)

<u>09/283,267</u>	<u>April 1, 1999</u>
(Application Serial No.)	(Filing Date)

CLAIM FOR BENEFIT OF EARLIER U.S./PCT APPLICATION(S)

I hereby claim the benefit under Title 35, United States Code, §120 of any earlier United States application(s) or PCT international application(s) designating the United States listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the earlier application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date(s) of the earlier application(s) and the national or PCT international filing date of this application. As to subject matter of this application which is common to my earlier application(s), if any, described below, I do not know and do not believe that the same was known or used by others in the United States or patented or described in a printed publication in any country before my invention thereof, or patented or described in a printed publication in any country or in public use or on sale in the United States more than one year prior to the date(s) of said earlier application(s), or first patented or caused to be patented or made the subject of an inventor's certificate by me or my legal representatives or assigns in a country foreign to the United States prior to the date(s) of said earlier application(s) on an application filed more than twelve months (six months if this application is for a design) before the filing of said earlier application(s); and I acknowledge that no application for patent or inventor's certificate on said subject matter has been filed by me or my representatives or assigns in any country foreign to the United States except those identified herein.

[illegible]

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorneys and/or agents to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

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Jane E. Remillard	Reg. No. 38,872	Debra J. Milasincic	Reg. No. 46,931
Jeremiah Lynch	Reg. No. 17,425	David R. Burns	Reg. No. 46,590
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Wherefore I petition that letters patent be granted to me for the invention or discovery described and claimed in the attached specification and claims, and hereby subscribe my name to said specification and claims and to the foregoing declaration, power of attorney, and this petition.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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